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ON THE RADIOACTIVE CONTAMINATION OF THE SEA

Annual Report 1967

prepared by M. BERNHARD

1969



CNEN Report No. RT/BIO (68) 60

Work performed at the
Laboratorio per lo studio della contaminazione radioattiva del mare

CNEN-EURATOM
Fiascherino, La Spezia, Italy

Association No. 024-63-2 BIAI

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 Annual Report 1967

Association : European Atomic Energy Community — EURATOM
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CNEN Report No. RT/BIO (68) 60
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The Fifth Annual Report of the CNEN-EURATOM Contract of Association
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ABSTRACT

The Fifth Annual Report of the CNEN-EURATOM Contract of Association is presented. The program laid down in this contract calls for studies of the factors which influence the uptake, accumulation and loss of radioisotopes by marine organisms. The program is divided into two parts:

- a) a survey of environmental elements and factors in a sampling area, and
- b) experiments on the influence of environmental factors on the uptake, accumulation and loss of radioisotopes by marine organisms in relation to the data obtained in the survey.

The task of carrying out this program has been divided between six groups: CHEMISTRY, BOTANY, ZOOPLANKTON, MICROBIOLOGY, FISHERIES, BIOLOGY and SPECIAL DEVELOPMENTS.

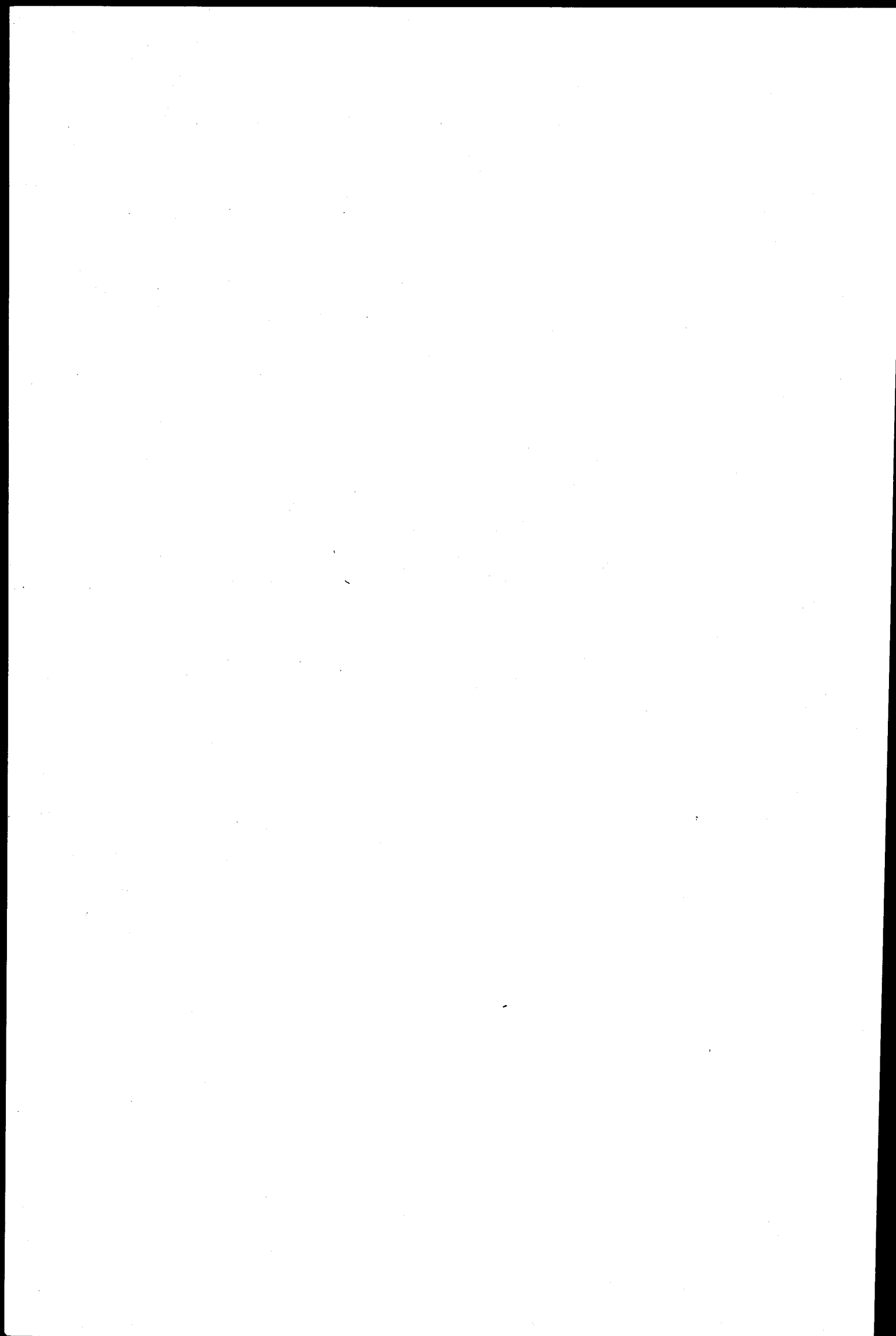
An account is given of the results obtained by these groups in 1967.

KEYWORDS

SEA
ENVIRONMENT
HYDROLOGY
RADIOISOTOPES
CONTAMINATION
DISTRIBUTION
ENRICHMENT
ALGAE
MICROORGANISMS
FISH
INVERTEBRATES
MEASUREMENT
ANALYSIS

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CHEMICAL ENVIRONMENTAL FACTORS IN MARINE (*)

RADIOCONTAMINATION

As in previous years the Chemistry Group has focussed its attention mainly on two lines of research:

- a) the distribution of stable elements which possess radioisotopes of importance in the field of marine contamination;
- b) the distribution of plant nutrients which may indirectly influence the accumulation of radioisotopes.

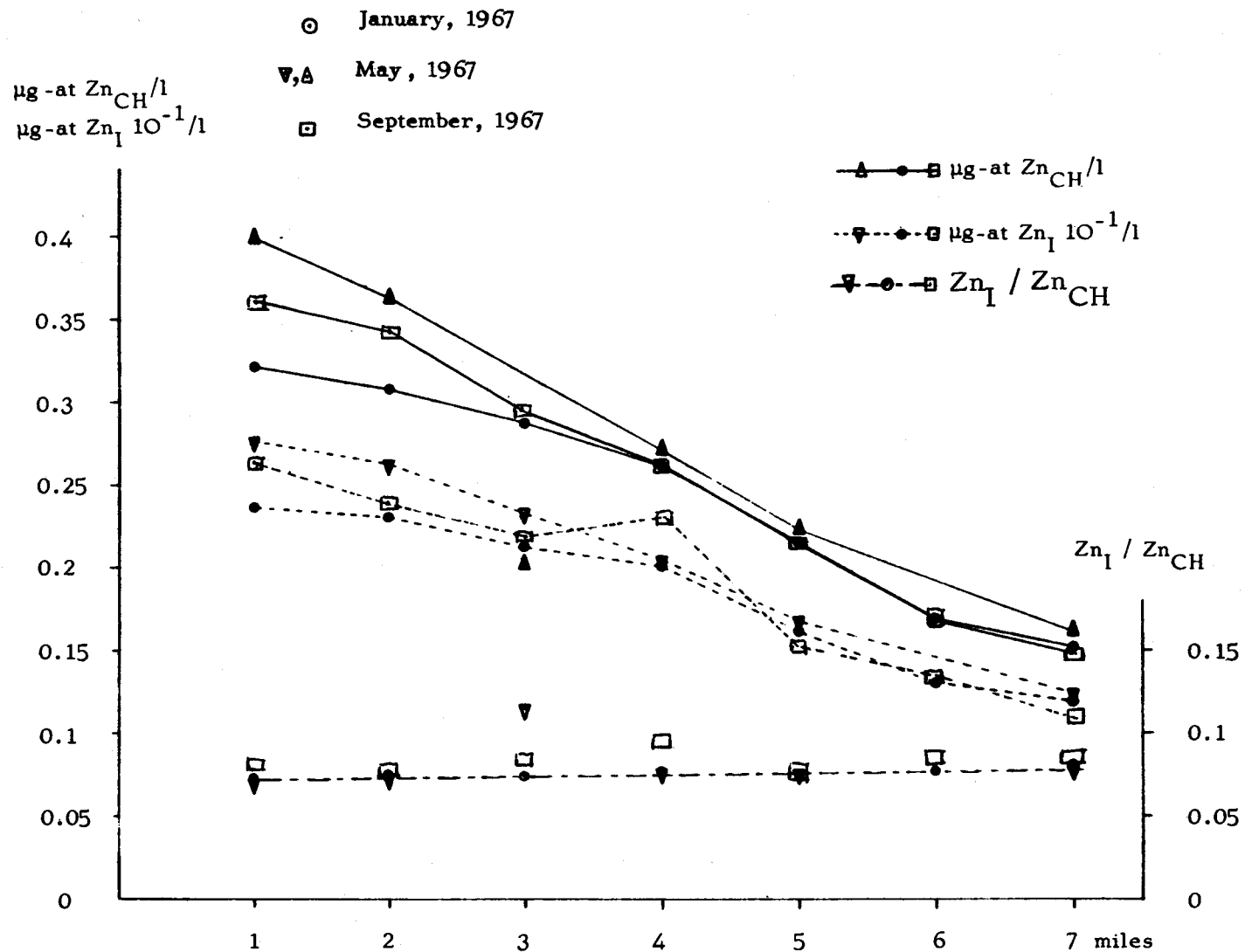
The steadily increasing evidence on the importance of physico-chemical states for the prediction of the behaviour of practically all radionuclides has enabled the group to increase its activity in the research of the different possible physico-chemical states of zinc in seawater.

Additional data on the vertical and horizontal distribution of total and ionic zinc have been obtained. Samples collected at 20 m depth horizontal to the sampling zone and vertically in the sampling zone confirm earlier observations (see Annual Report 1966): i.e., total Zn extracted with Chelex and ionic Zn determined with the polarograph decrease with distance from the coast (Fig. 1).

(*) Manuscript received on 27 January 1969.

Horizontal distribution of ionic and total Zn in the Ligurian Sea.

Fig. 1



The vertical distribution in the sampling zone shows a remarkable constancy of the values for different depth at different periods (Fig. 2).

Most striking is the stability of the ratio $\frac{\text{ionic zinc}}{\text{total zinc}}$ which at a depth lower than 10 m is practically constant.

In order to see if this constancy also exists in the shore waters, the distribution of ionic and stable zinc has been studied in the Gulf of La Spezia including the mouth of the river Magra and the area in front of Palmaria island (Fig. 3).

This was done in order to study the introduction of Zn from the river Magra and link the data from the Gulf with those of the horizontal and vertical distribution of the sampling area.

As can be seen from Fig. 3, total zinc (Zn_{CH}) seems to decrease with the distance from the port of La Spezia (Points 1, 2, 3, 4 and 13, 14, 15), but relatively high zinc contents are also found at points 8, 9, 10 and 11.

The influence of high Zn content in the water of river Magra is probably responsible for the concentrations at points 5 and 6.

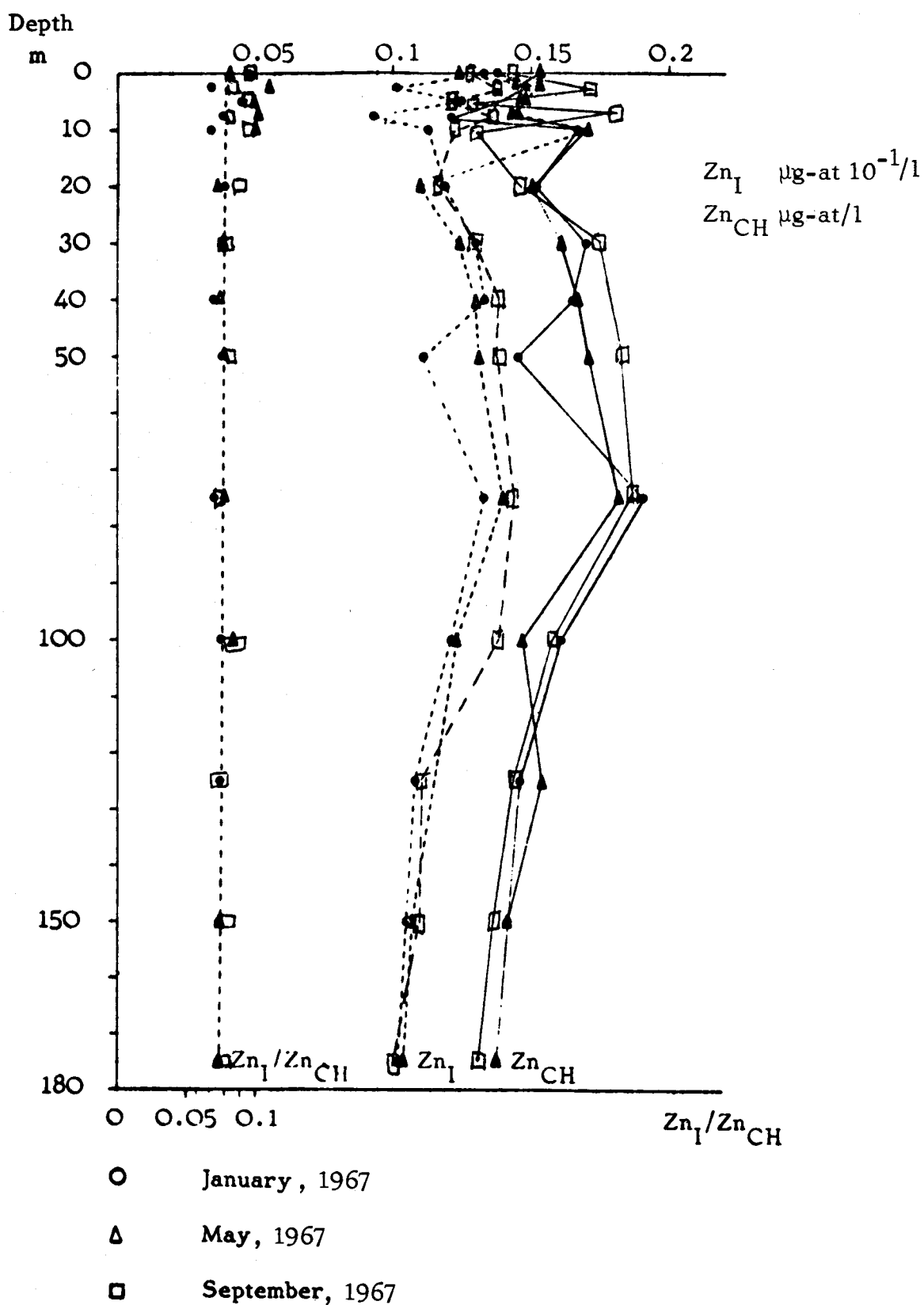


Fig. 2

Vertical distribution of ionic and total Zn in the Ligurian Sea.

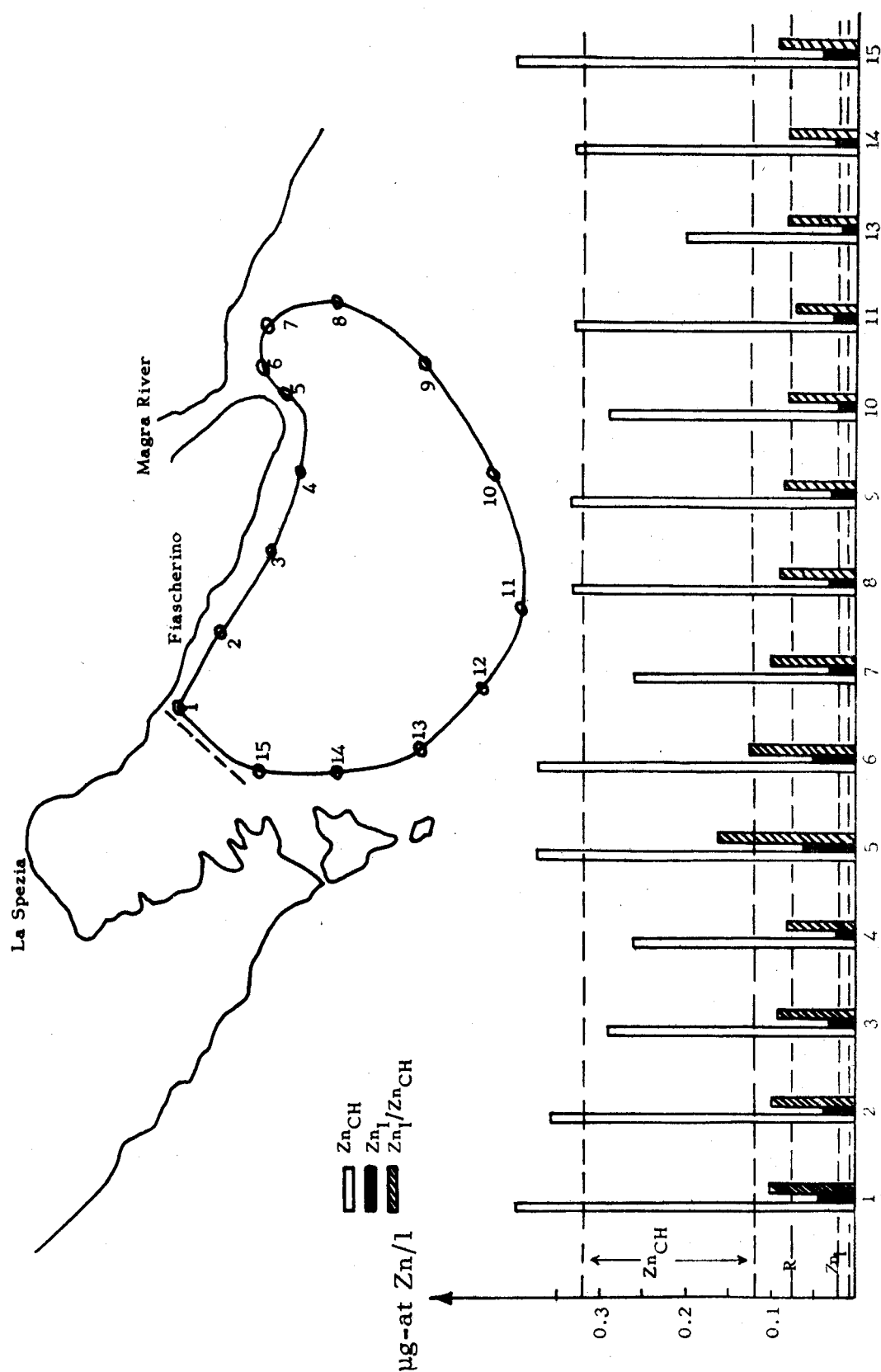


Fig. 3

Distribution of Zn in samples collected during a cruise in the Gulf of La Spezia (September, 1967).

In the Magra the following values were found ($\mu\text{g-at Zn/l}$)

| May, 1967 | Zn_{CH} | Zn_{I} | $\frac{\text{Zn}_{\text{I}}}{\text{Zn}_{\text{CH}}}$ |
|-------------------------|-------------------------|------------------------|--|
| Bridge "Aurelia" | 0.61 | 0.20 | 0.33 |
| Bridge "Bocca di Magra" | 0.61 | 0.16 | 0.27 |

However, the concentrations are determined by the current pattern in this area, and a better explanation for the observed values can be supplied in the future when the currents of the zone can be studied.

Results obtained previously on the uptake of Zn by ion exchange resins and also on the uptake and loss of Zn by algae suggested that the algae and also ion exchange resins taken up preferable ionic Zn. Following a suggestion of Dr. Branica a method (potentiostatic electrolysis) is under study which should enable us to determine whether the ionic radioactive Zn added to the seawater will be diluted in the ionic stable Zn, and how many of the radioactive ionic Zn atoms added will be exchanged with atoms present in complexed form in the seawater.

So far the influence of complexing agents and the influence of stable isotopes have been studied.

In order to automate and adapt the Zn-determinations to Autoanalyzer techniques, the determination of Zn with dithiozine has been set up.

Minimum quantity to be determined : 0.02 $\mu\text{g-at Zn/l}$. After finalization this technique will be used together with the newly developed apparatus which automatically goes through a complete cycle of changing, eluting and rechanging of ion exchange resins (see Special Developments Group).

The "Autoanalyzer" technique for the determination of N-Kjeldahl has been modified for use in seawater: 10 samples can be analyzed per hour.

At present the range extends from 5-200 $\mu\text{g-at N/l}$.

Simultaneous determination of NO_2 , NO_3 , and hydrolyzable total P-PO_4 have been analyzed aboard the "Odalisca" in order to try out these determinations at sea.

FIRST TROPHIC LEVEL OF THE FOOD CHAIN

In 1967 the Botany Group continued the work on the accumulation of stable and radioactive zinc¹⁾ and radioactive and stable phosphorous following the distribution of the stable and the radioactive isotope between the algae and the medium.

The accumulation of zinc by Phaeodactylum tricornutum was studied in the presence of chelates. If cysteine is added (see Fig. 4), the amount of stable and radioactive zinc accumulated is somewhat less than without cysteine. However, as in previous experiments, always more radioactive zinc than stable zinc is taken up.

An experiment designed to study the influence of the size of the algae population on the uptake of Zn showed that only the uptake of radioactive Zn is influenced by the population size (Fig. 5). The quantity of stable total Zn taken up is the same for different populations. These findings are contrary to those observed in P-uptake experiments, where for both stable and radioactive P a correlation between population size and amount of P taken up exist.

¹⁾ The first results of this work were presented at the Second National Symposium for Radioecology, Ann Arbor, April 1967.

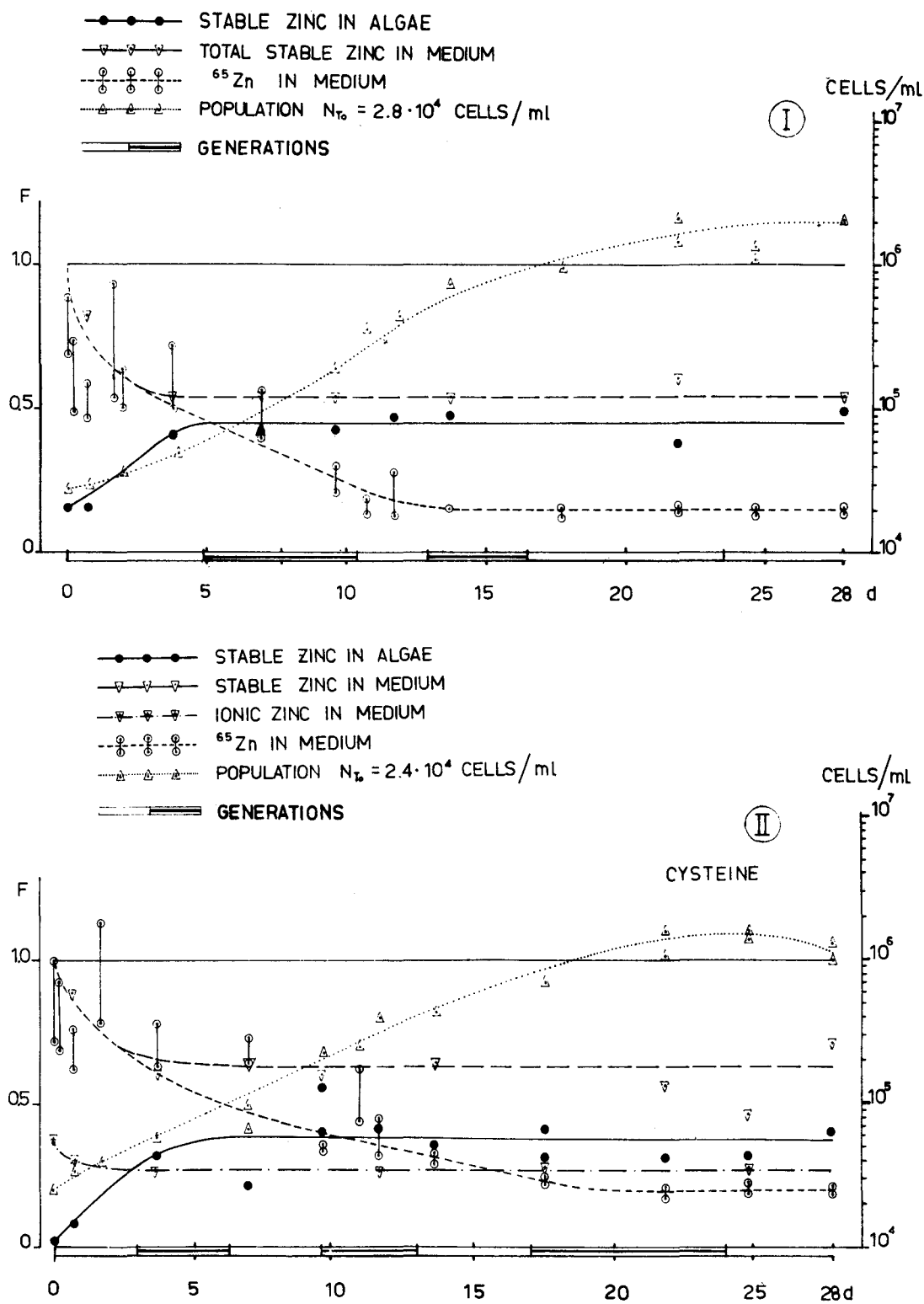


Fig. 4

Uptake of ^{65}Zn and stable zinc by Phaeodactylum tricornutum in successive days (d) and generations from natural seawater (I) and with addition of stable zinc and cysteine to the medium (II).

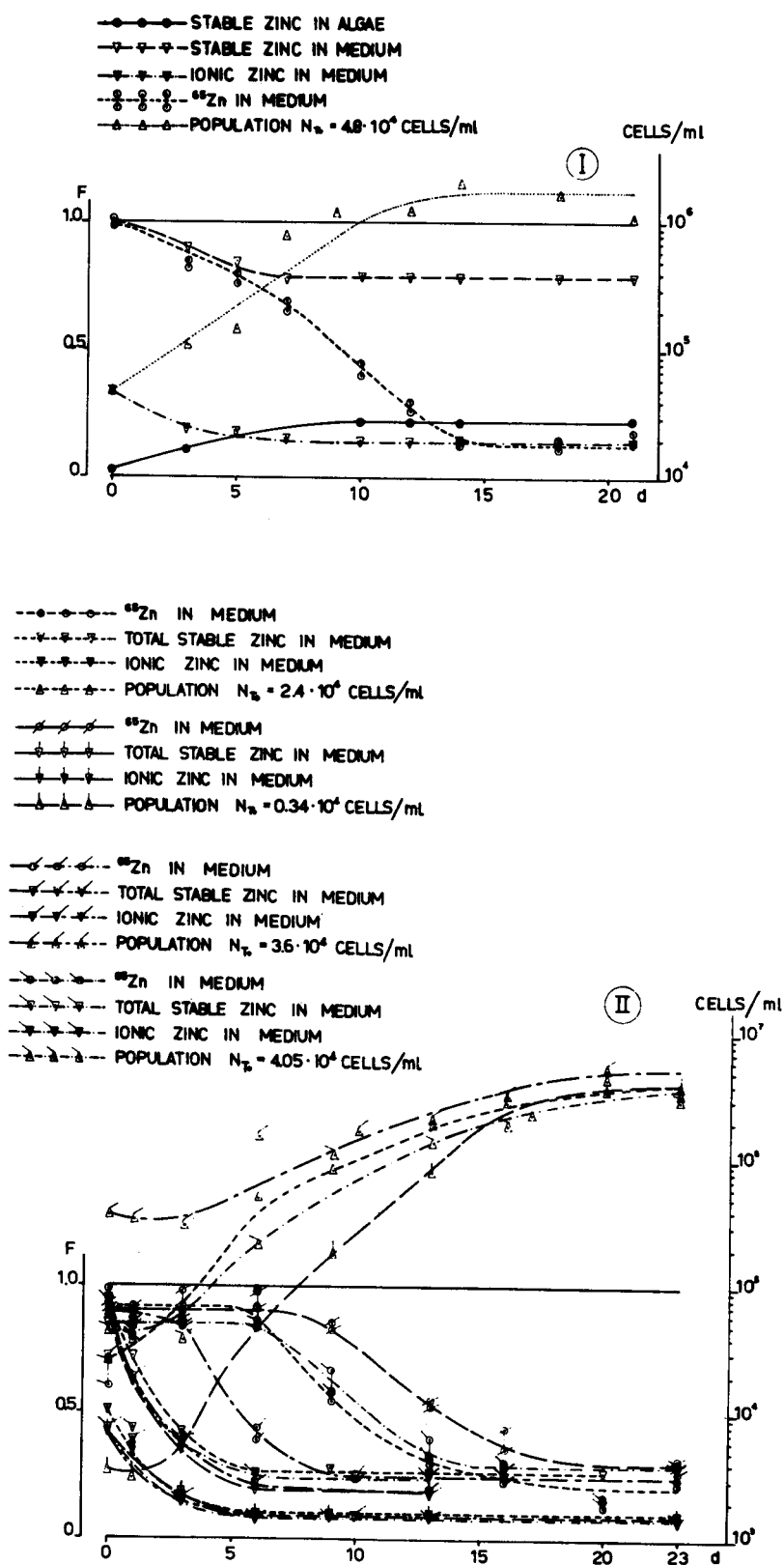


Fig. 5

Relation between the stable and radioactive zinc taken up and the population size. Zinc was partially complexed with Cysteine. Algae populations from experiment I were used for the experiment II.

In fact it could be observed that contrary to the accumulation of zinc, the amount of ^{32}P accumulated is related to the population size and that a high enough population can in effect take up all the ^{32}P added (see Figs. 10 and 11 of the Annual Report 1966).

The influence of a change in the concentration of stable phosphorus in the medium and the uptake of stable and radioactive phosphorus was examined. The results show that the amount accumulated depends on the concentration of the stable phosphate in the medium into which the algae have been transferred. Furthermore, the algae take up stable phosphate at the same rate as the radioactive phosphate, so it seems that the uptake mechanism of phosphate is different from that of zinc.

In order to study the exchange between ^{65}Zn complexed to EDTA and stable Zn and vice versa, uptake experiments have been carried out in which ^{65}Zn was first added to EDTA and followed by stable ionic Zn.

This design was compared with one in which stable Zn was first complexed with EDTA and then ^{65}Zn added. Surprisingly both experiments gave the same results (Fig. 6).

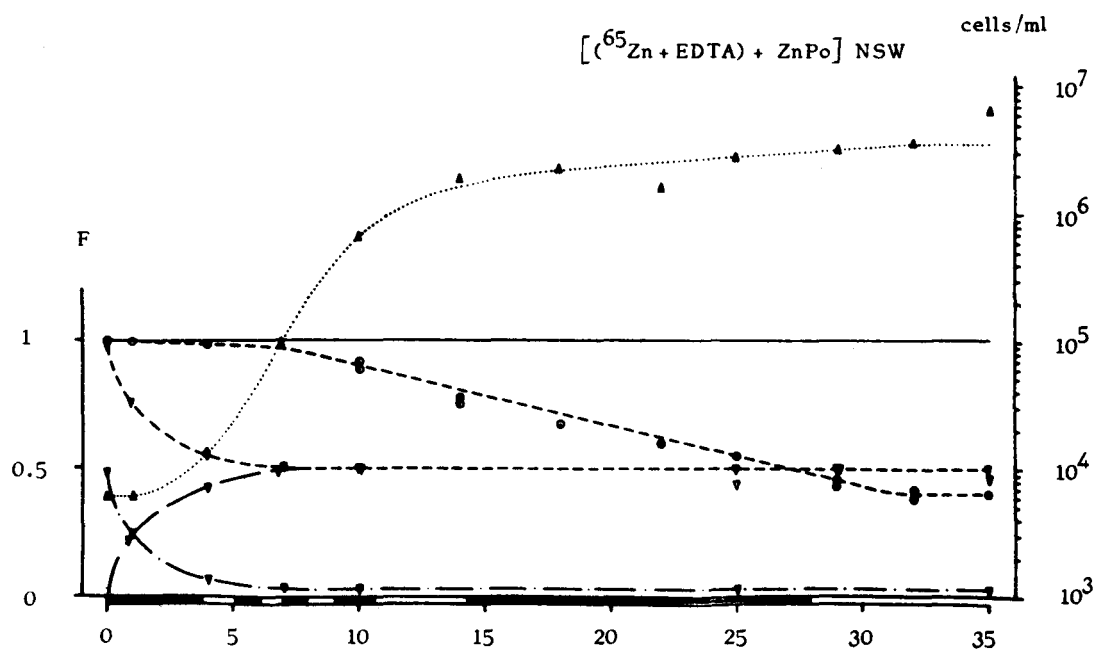
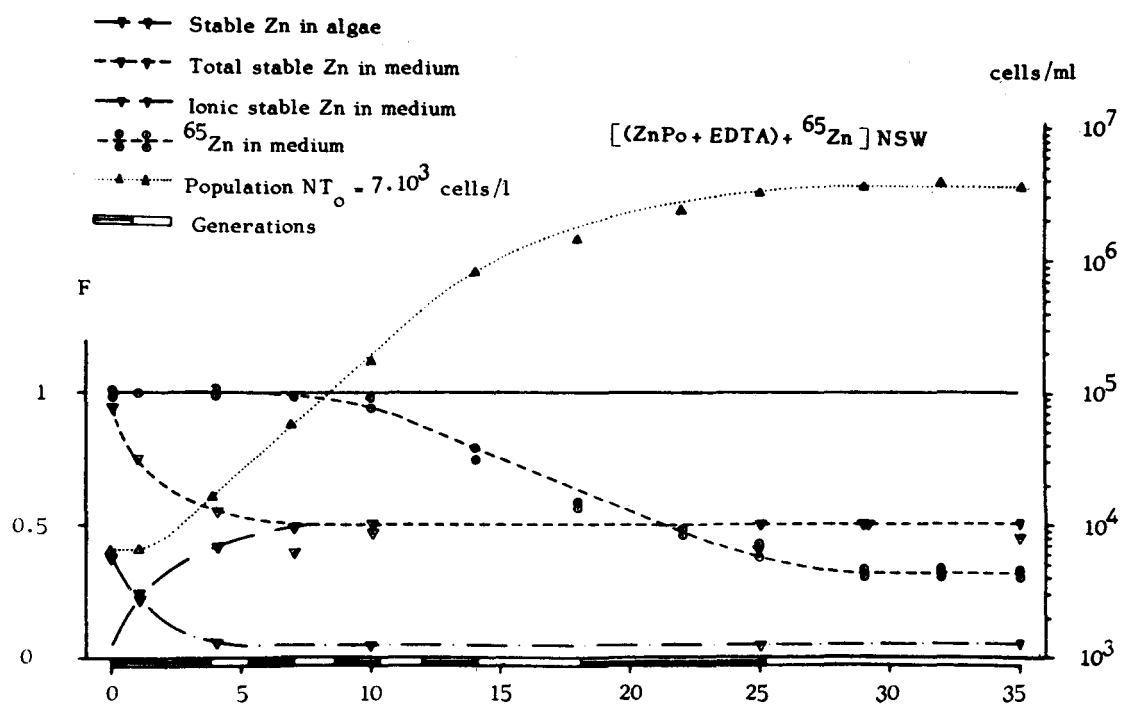


Fig. 6

Comparison between ^{65}Zn not complexed with EDTA, and ^{65}Zn all complexed with EDTA. In both cases the amount of EDTA was sufficient to complex the stable zinc at 50%.

A comparison between one experiment in which the algae were transferred from 1.64 to 1.64 $\mu\text{g-at Zn/l}$ and one in which the stable Zn changed from 1.64 to 4.92 $\mu\text{g-at Zn/l}$ showed that in the latter case proportionally less stable and less radioactive Zn was taken up (Fig. 7).

Summarizing the observations of all experiments carried out so far we obtain Fig. 8.

As can be seen, there exists a loose correlation between the Zn concentration in the medium and the Zn concentration in the algae.

Summarizing the results obtained in the uptake experiments up to now, it seemed that the algae and the resins can fractionate the zinc present in seawater. In order to predict with a chemical method the quantities and physico-chemical states taken up by the algae, it will be necessary to develop chemical methods capable of determining the same fraction of the zinc present in the seawater which is available to the algae.

For the phosphorus on the other hand, the chemical methods at hand seemed to determine the same amount of phosphorus which is also

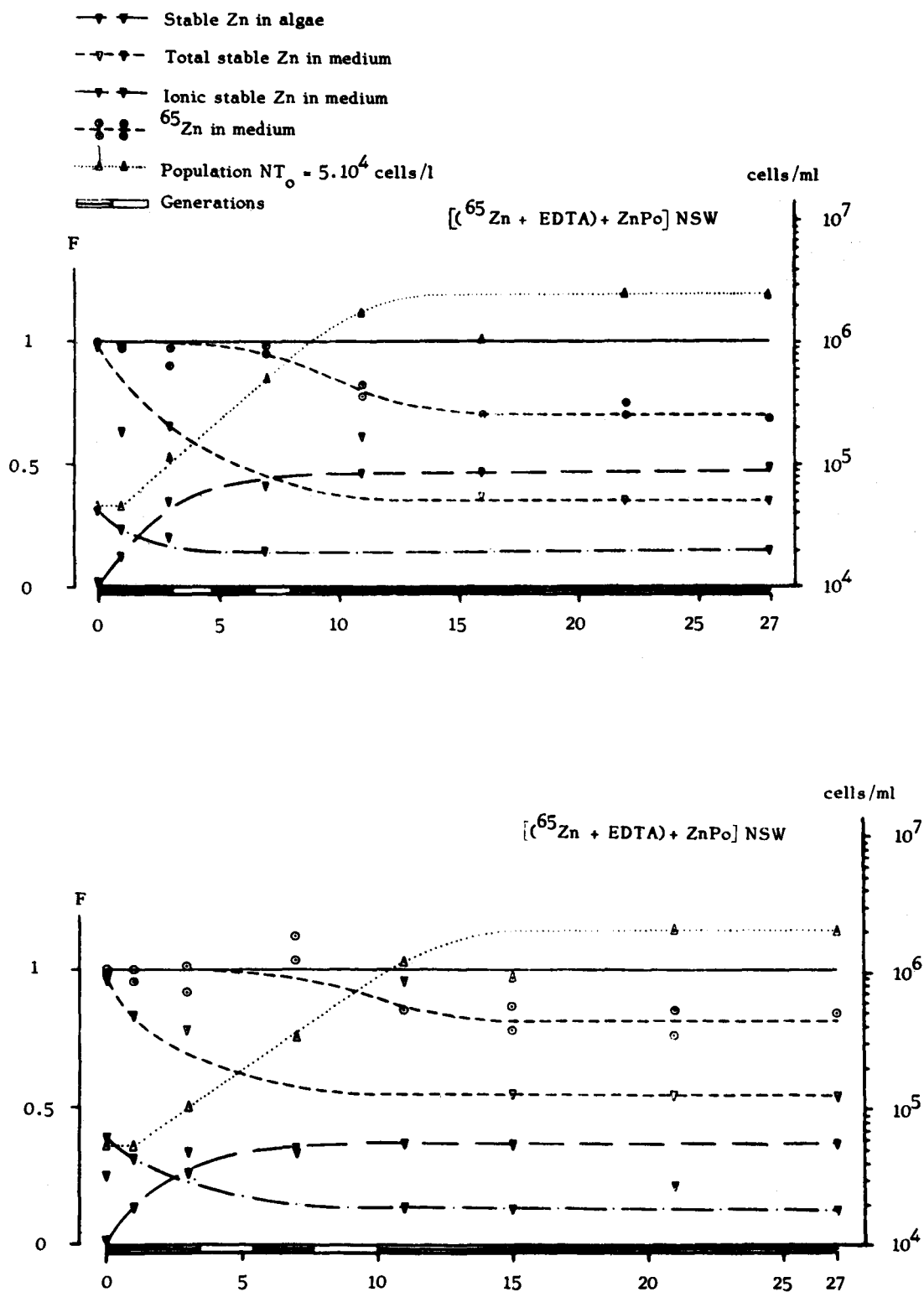


Fig. 7

Comparison between Zn uptake in population grown in $1.64 \mu\text{g-at Zn/l}$ and then transferred to a medium of same zinc content and one population grown in $1.64 \mu\text{g-at Zn/l}$ and/ then transferred to a medium of $4.92 \mu\text{g-at Zn/l}$.

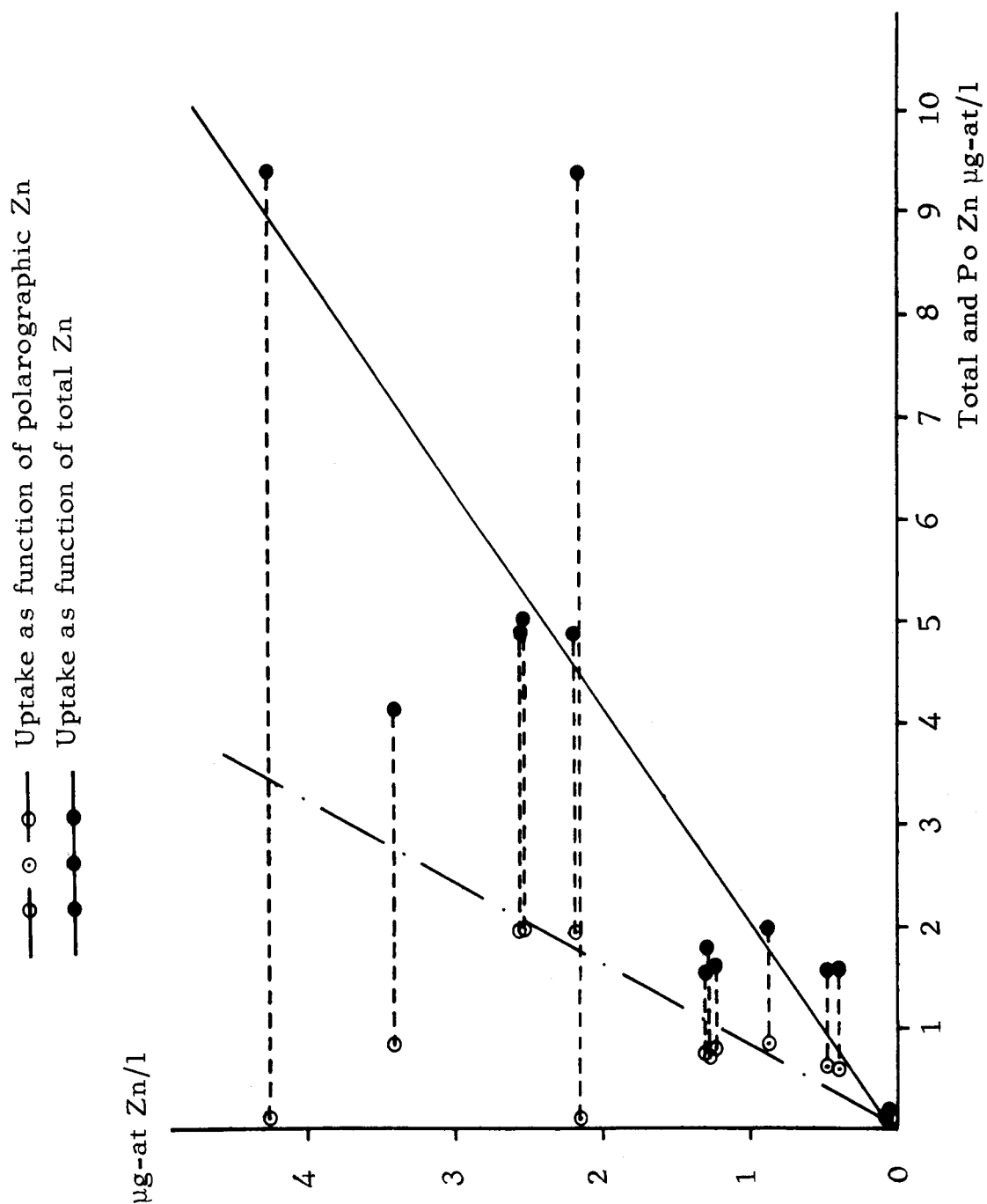


Fig. 8

Summary of the results obtained in all experiments.

Relations between the zinc content in the algae and the polarographic and total zinc in the medium.

available to the algae, and hence no difference is encountered in the distribution of radioactive and stable phosphorus between the medium and the algae.

In order to relate laboratory experiments to the natural environment, a comparison between the Zn concentration of a natural population (bloom), consisting mainly of Chaetoceros and Ceratium species, showed that algae (Phaeodactylum) from the culture enriched the Zn to 30 times the concentration of the natural population, although the Zn concentration in the culture was about the same as in the water under natural conditions ($0.216 \mu\text{g-at Zn}_T/\text{l}$).

Experiments carried out to kill algae with UV have shown that the algae examined exhibit increasing sensitivity from Phaeodactylum tricornutum, Nitzschia closterium, Nitzschia seriata, Platymonas suecica to Coccolithus huxleyi.

UV-killed algae will be used to study the uptake of radioisotopes in order to separate growth and exchange accumulation from adsorption.

A method has been set up to determine simultaneously stable carbon and ^{14}C utilizing the F/M gas-chromatograph and a plastic scintillation counter. This technique will be used for investigation on primary productivity.

Continuing the studies concerning the storage of algae culture at low temperature under different experimental conditions, they have shown that only Phaeodactylum tricornutum survives storage for 6 months at -20°C .

Since ion regulation seems to play an important role in the accumulation of elements and very little is known about the ion regulation of phytoplankton in general, preliminary experiments have been carried out on the Na-regulation by Platymonas and Phaeodactylum in seawater diluted down to 1/10 normal seawater.

The results obtained have shown that the Na-concentration in the algae decreases with salinity. Therefore, it seems likely that the algae maintain the cell turgor by regulating sodium.

HETEROTROPHIC LEVELS OF THE MICROORGANISMS

The main efforts of the Microbiology Group were directed toward the construction of an artificial key based on a set of tests administered by Melchiorri-Santolini (see previous Reports) to 229 strains of culturable bacteria (Fig. 9). The strains were then subjected to the tests chosen for the construction of the key in order to determine whether the response to the tests changed as a result of adaptation to laboratory culture conditions. As can be seen from Table 1, a maximum of 20% of the strains changed their response to the tests. Since more than two tests are given for most decisions the probability of a wrong classification is of course, smaller.

The uptake of ^{32}P by the lambda strain has been studied utilizing an autoradiographic technique. A comparison between UV-killed bacteria and bacteria in lag and log-phase showed that a marked difference in the number of spots between these three conditions exists (Table 2).

Classification key for marine microorganisms.

Fig. 9

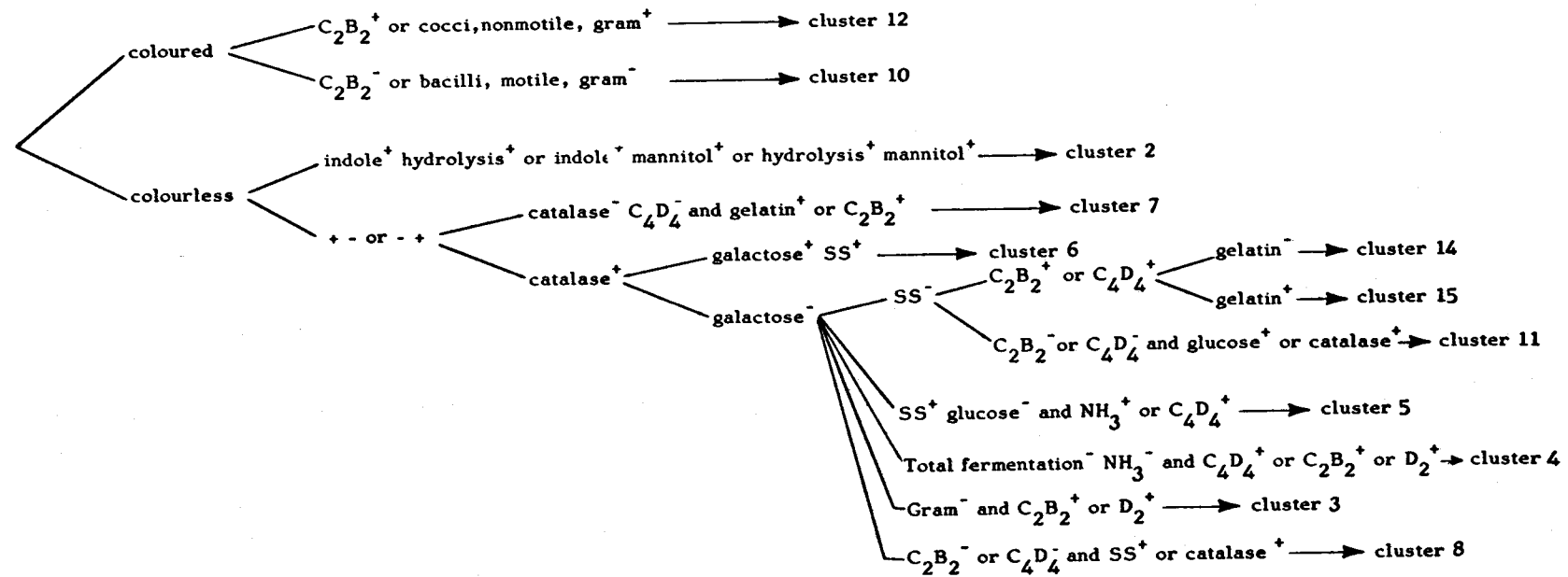


TABLE 1

Constancy of responses to tests administrated to microorganisms
in 1960-62 and in 1967

| Test | Number of strains examined | Strains changed | Percentage changed |
|-------------------------------|-------------------------------|-----------------|-----------------------|
| | (1) | | |
| H ₂ O | 222 | 50 | 23 |
| INDOLE | 222 | 12 | 5 |
| NH ₃ | 222 | 26 | 12 |
| NO ₂ ⁻ | 222 | 51 | 23 |
| GELATIN | 222 | 38 | 17 |
| GLUCOSE | 222 | 37 | 16 |
| GALACTOSE | 222 | 19 | 9 |
| MANNITOL | 222 | 33 | 15 |
| C ₂ B ₂ | 222 | 26 | 12 |
| C ₄ D ₄ | 222 | 28 | 13 |
| D ₂ | 222 | 25 | 11 |
| SS | 222 | 30 | 13 |
| HYDROLYSIS | 222 | 38 | 17 |
| CATALASE | 222 | 16 | 7 |
| TOTAL FERM. | 222 | 36 | 16 |

(1)

Seven strains are lost

TABLE 2

Uptake of ^{32}P by strain lambda

| | Total spots/ml | Net spots/ml | Plate counts (cells/ml) |
|---------------------|----------------|--------------|-------------------------|
| UV-killed bacteria | 45 | 10 | 0 |
| lag-phase bacteria | 206 | 171 | 300 |
| log-phase bacteria | 448 | 413 | 400 |
| Blank (UV seawater) | 35 | -- | 0 |

The horizontal and vertical distribution of bacteria from the coast towards and in Station I was analyzed using different media and different plating techniques (Table 3).

Spreading the bacteria on agar surface a higher number of colonies were obtained than when the "double layer" technique was used.

The number of colonies decreased also when non-enriched media were employed. Great differences between samples taken from different depths seemed to indicate a very heterogeneous microdistribution of viable bacteria.

The mineralization of phosphate by bacteria present in seawater was studied in collaboration with the Chemistry Group. An unknown bacteria strain isolated from seawater which had been stored for some time in the laboratory was used. This strain mineralized about 80% of the organic phosphorus present after 10 days (Fig. 10). Similar results were obtained with a strain (λ) from our culture collection, except that it took a much longer time before the strain mineralized a considerable amount of the organic phosphorus.

Horizontal distribution from Porto Venere to Station A

TABLE 3

| Plate counts (cells/ml) | | | | | | | MPN cells/ml |
|--|----------------------------|------------|-----------------|--|--------------------------|--|-----------------|
| Station (20 m depth) miles off coast | Double layer | | | Spreading on surface | | | |
| | OZ-agar | SW-agar | SW-glucose-agar | OZ-agar | SW-agar | SW-glucose-agar | |
| 1 | 27 75 | | 2 1 | 80 25 | 0 | 15 confluent growth | 24 |
| 2 | 109 confluent growth | 1 0 | | 35 | 5 0 | 0 0 | |
| 3 | 43 72 | 4 0 | 2 | confluent growth | 0 0 | 0 0 | 170 |
| 4 | 48 43 | 3 1 | 0 1 | confluent growth 250 | 5 0 | 0 confluent growth | |
| 5 | confluent growth 96 | 3 2 | 0 0 | 50 30 | 0 0 | 0 0 | 160 |
| 6 | 50 20 | 8 6 | | confluent growth confluent growth | | confluent growth confluent growth | |
| 7 | 16 22 | 5 0 | 0 0 | confluent growth 35 | confluent growth 0 | 0 | 35 |

(to be continued)

Vertical distribution at Station A

Plate counts (cells/ml)

| Depth m | Double layer | | SW-glucose-agar | Spreading on surface | | |
|------------|--------------|---------|-----------------|----------------------|---------------------|------------------|
| | OZ-agar | SW-agar | | OZ-agar | SW-agar | SW-glucose-agar |
| 2 | 50 | 2 | | confluent growth | 5 | confluent growth |
| | 150 | 0 | | confluent growth | 0 | 0 |
| 5 | 25 | 4 | | 250 | | |
| | 60 | 1 | | 125 | | |
| 10 | 16 | 4 | | confluent growth | 0 | 5 |
| | 18 | 7 | | 30 | 0 | 0 |
| 20 | 22 | 5 | 0 | confluent growth | confluent growth | 0 |
| | 16 | 0 | 0 | 35 | 0 | |
| 30 | 36 | 3 | | confluent growth | | |
| | 50 | 0 | | 225 | | |
| 50 | 18 | 1 | | confluent growth | 5 | 0 |
| | 17 | 0 | | 10 | 0 | |
| 75 | 2 | 0 | | confluent growth | | |
| | 12 | 0 | | 0 | | |
| 100 | 5 | 0 | | 5 | 0 | 0 |
| | 8 | 0 | | 0 | 0 | 0 |
| 125 | 3 | 0 | | 5 | 0 | |
| | 3 | 0 | | 0 | 0 | |
| 150 | 8 | 4 | | 5 | 5 | |
| | 25 | 0 | | 5 | | |
| 175 | 250 | 1 | | 5 | 5 | 5 |
| | 28 | 5 | | 5 | 0 | 10 |

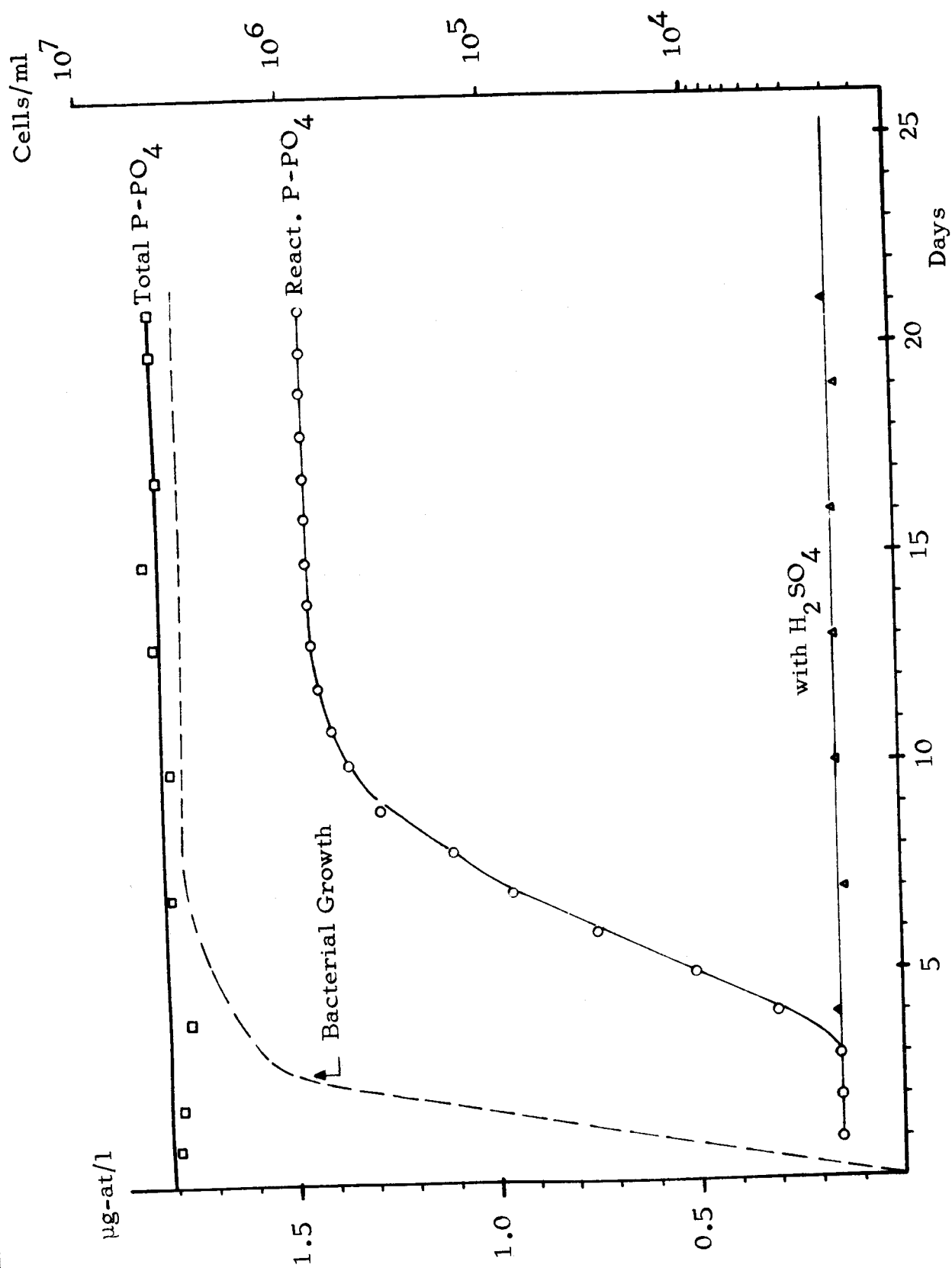


Fig. 10

Mineralization of β -glycero-phosphate added to natural seawater (1.5 $\mu\text{g-at/l}$) by a bacteria strain.

FIRST HETEROTROPHIC LEVEL OF THE FOOD CHAIN

During the period under discussion the Zooplankton Group directed its main efforts to the study of the life cycle of predominant copepod species and to obtaining data on catching performance and filtration efficiency of the plankton nets. This information is necessary for preparing the ground for the experimentation with radioisotopes on ecologically significant zooplankton species.

As pointed out in earlier Annual Reports, the sampling techniques known at present are not sufficiently reliable for supplying data which are significant and representative enough to determine which are the ecologically important species.

The catching performance of plankton nets is imperfectly known and no identification keys exist for the numerically very important larvae forms of zooplankton species.

In order to determine the species to which the numerous nauplii and copepods found in the sampling zone, an arbitrary key for the most frequent nauplii and copepodites was made. The nauplii and copepodites which were predominant in samples taken at sea were isolated and transferred to culture media. At different time intervals samples were taken from these culture and fixed.

Furthermore, eggs are isolated from adults and again fixed at different time intervals in order to obtain different larval stages (Fig. 11).

For identification the nauplii were immobilized with CO_2 .

Preliminary results show that this technique is promising.

It will enable us to investigate also the distribution of nauplii and copepodites, a very important group of plankton organisms which is hardly ever considered in plankton surveys, since keys for their classification are not available.

Experiments on the catching performance of high-speed plankton nets (Delfino) have been continued. The influence of towing speed (2-7 kn) and pore size of the nets (71 and 180 μ) was compared.

The results obtained showed that with increasing speed more copepods are caught. However, in the 71 μ nets twice as many organisms wider than 180 μ are caught than in 180 μ nets. This result is not easy to interpret, since filtering experiments with fixed plankton samples have shown that organisms of 180 μ width do not pass through 180 μ pores.

After several experiments in which the pore size and filtering conditions were varied had not given reproducible results (Fig. 12), we had to

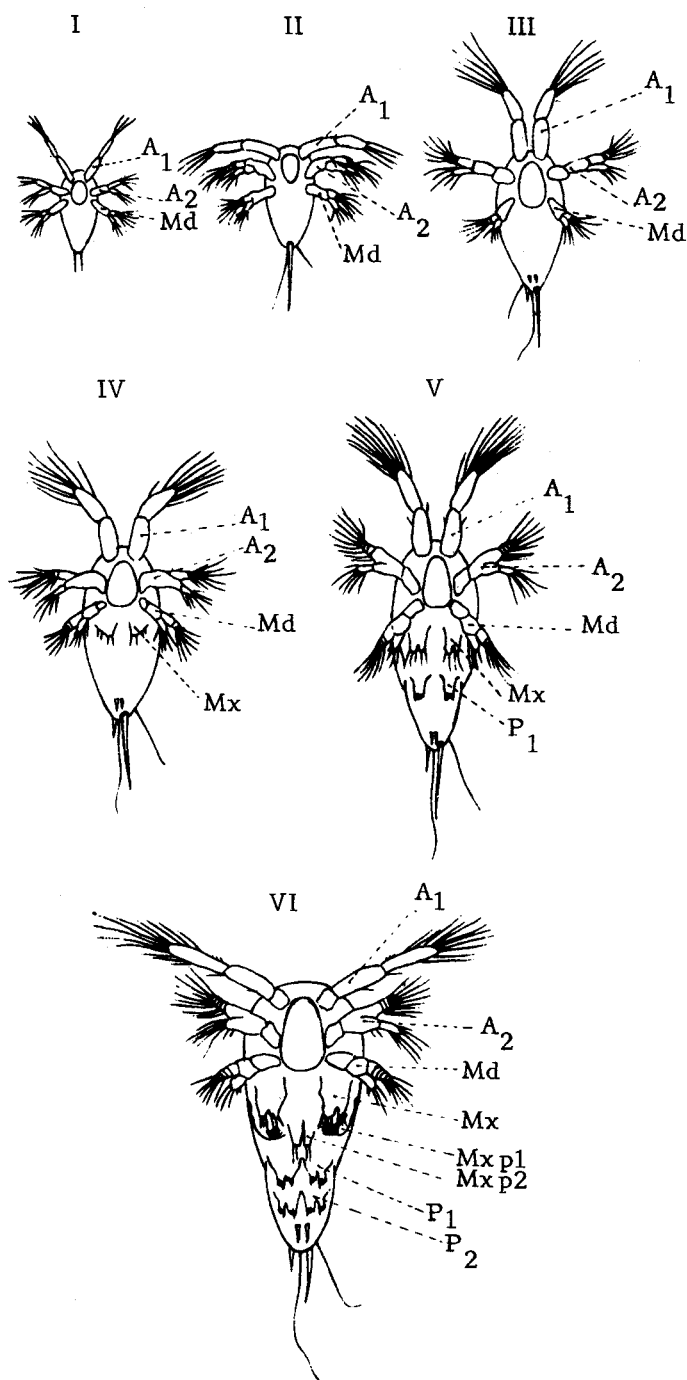


Fig. 11

Different larval stages of a copepod (Isias clavipes).

A₁ = 1st antenna

A₂ = 2nd antenna

Md = mandible

Mx = maxilla

Mxp₁ = 1st maxilliped

Mxp₂ = 2nd maxilliped

P₁ = 1st leg

P₂ = 2nd leg

% of organisms
before filtration

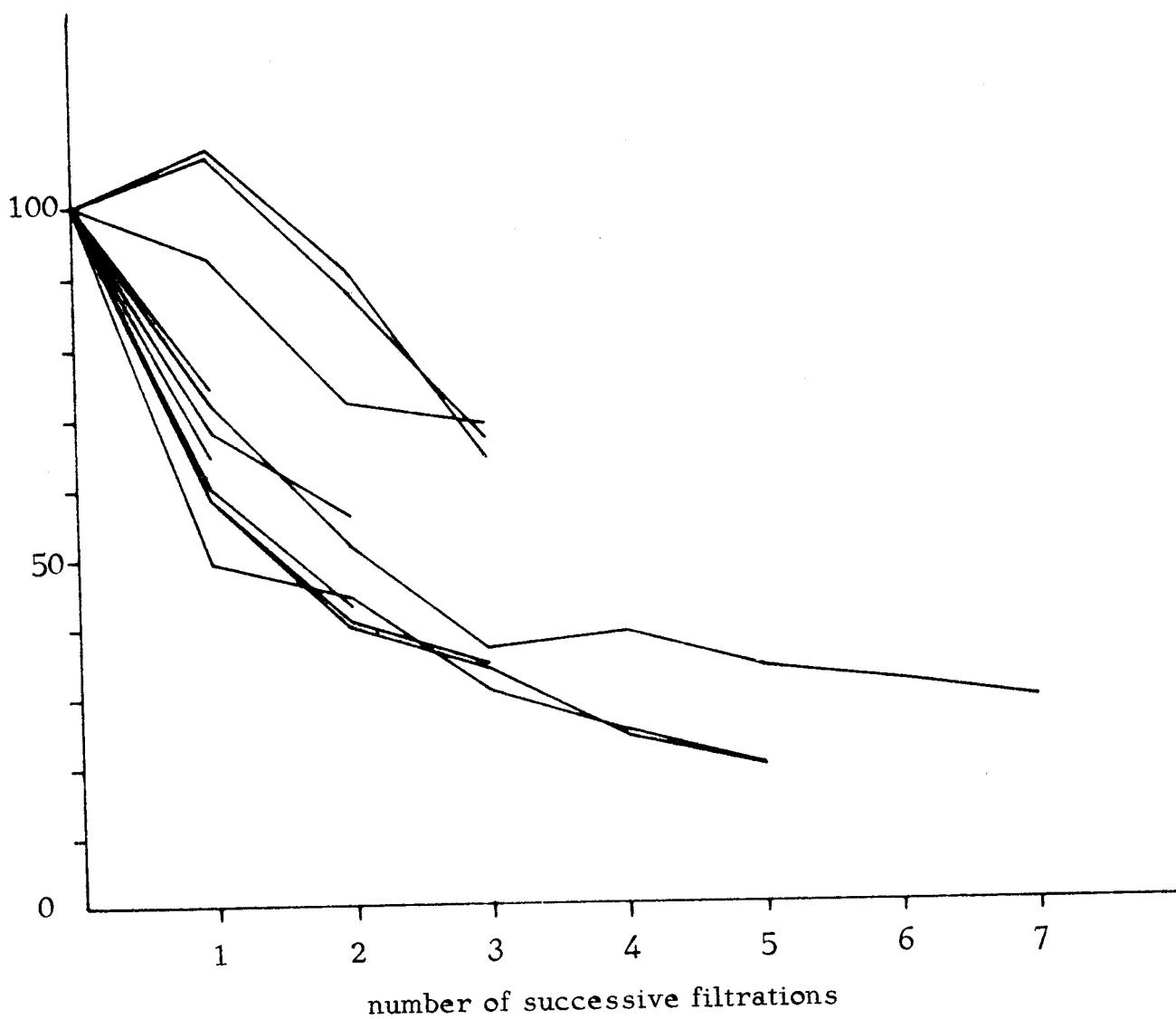


Fig. 12

Summary of experiments on successive filtrations of samples taken
with a 180- μ -pore size-net without sizing.

return to the original technique, determining the size of the zooplankton organisms with a grid, and count only those which are wider than the pore size of the net. For 180 μ nets the critical diameter of fixed plankton is about 180 μ , so only those organisms were counted the bodies which were wider than 200 μ .

In this way it was possible to obtain reproducible results even after several filtrations through a 180 μ pore size net (Fig. 13).

If a 71 pore size net is used, reproducible results are obtained if only those organisms which are wider than 85 μ (body diameter) are counted.

However, it may be realized that these limits are valid only for fixed plankton. The actual minimum escape size of live plankton organisms may be larger.

The experiments on the rearing conditions have been continued, but so far none of the factors important for optimum conditions has been discovered.

P; Zn, C and N have been determined in Calanus minor and Clausocalanus from natural population and Euterpina acutifrons from a laboratory culture.

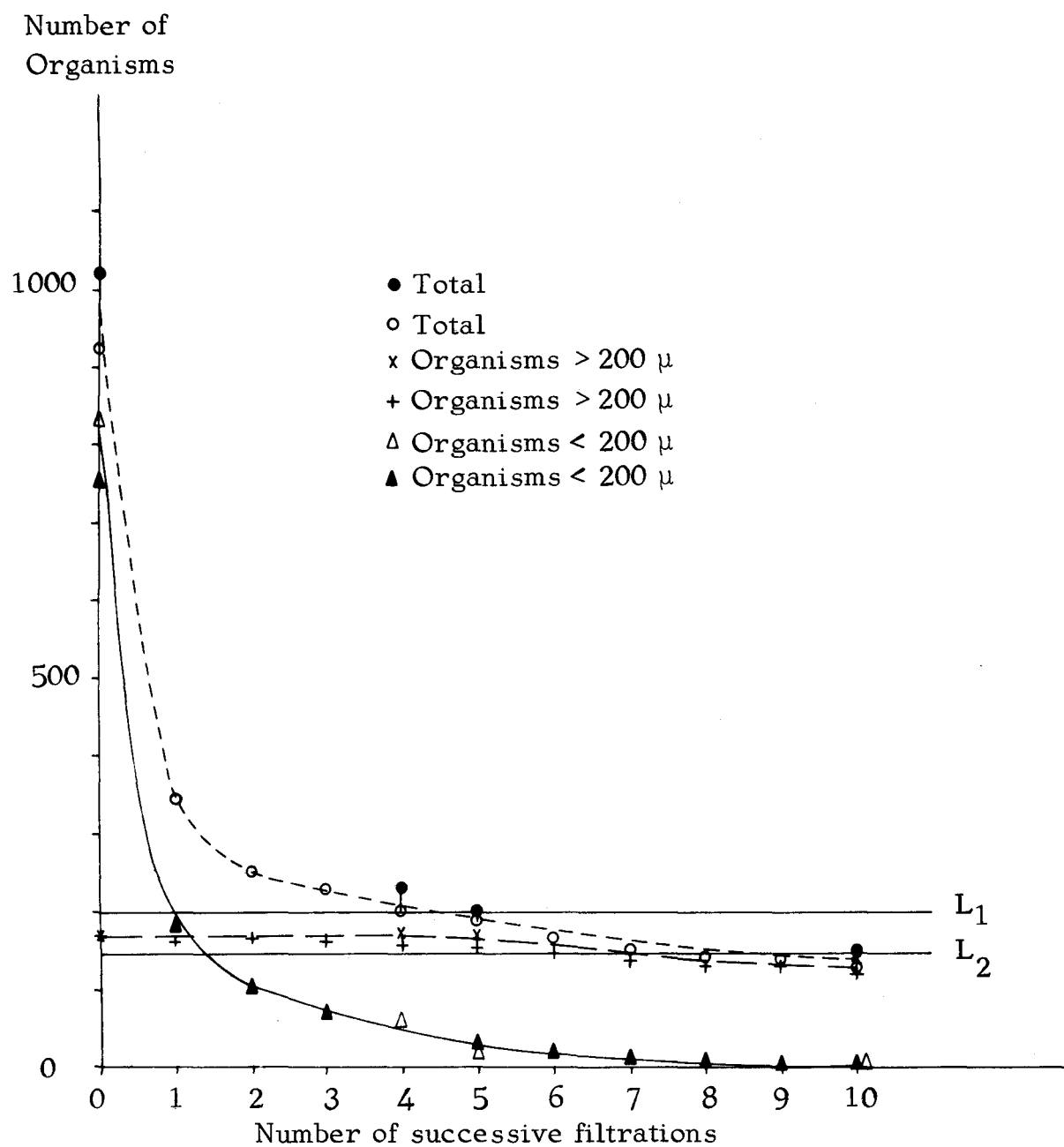


Fig. 13

Filtrations vs. sizing in successive filtrations.

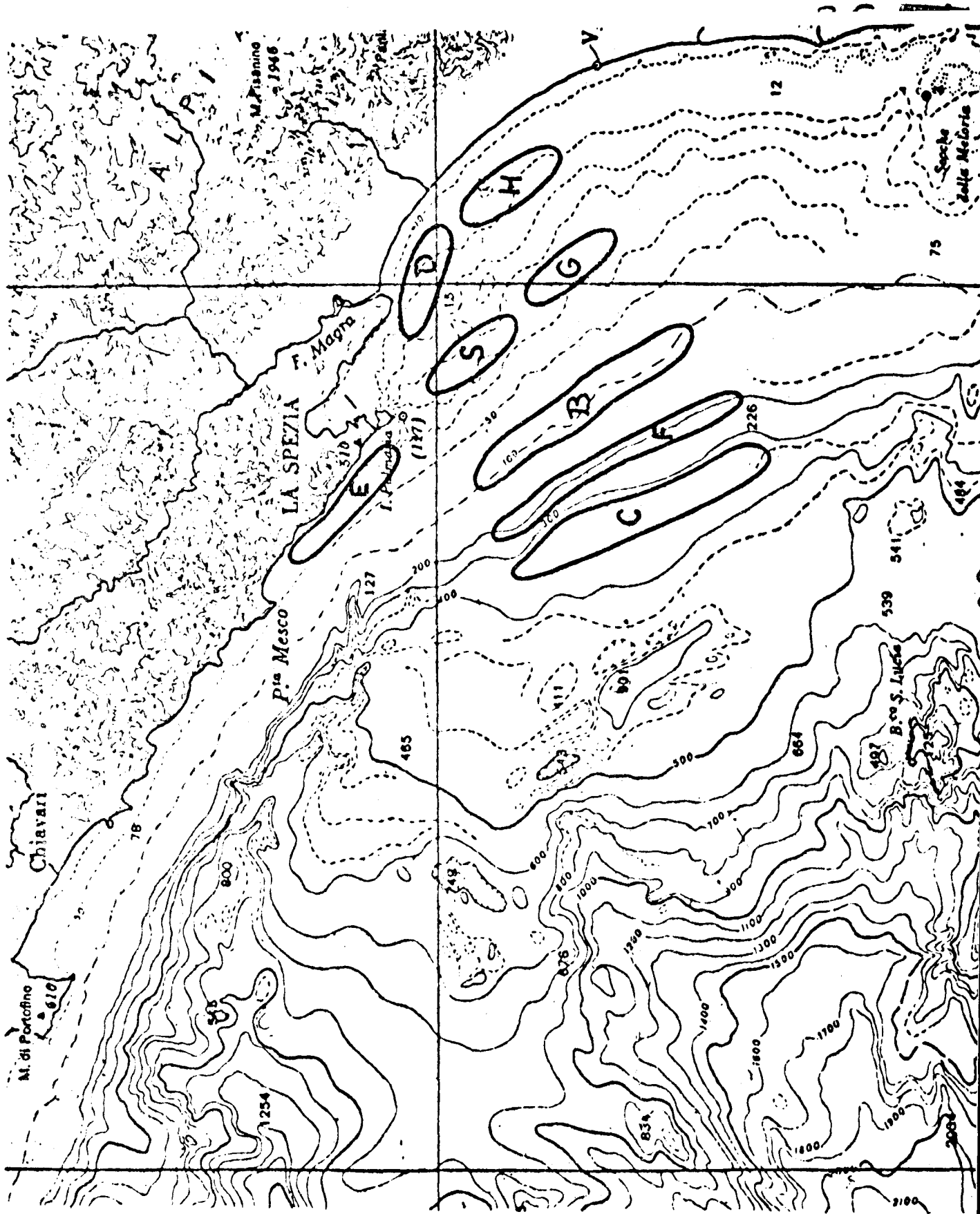


Fig. 14

Fishing zones in 1967.

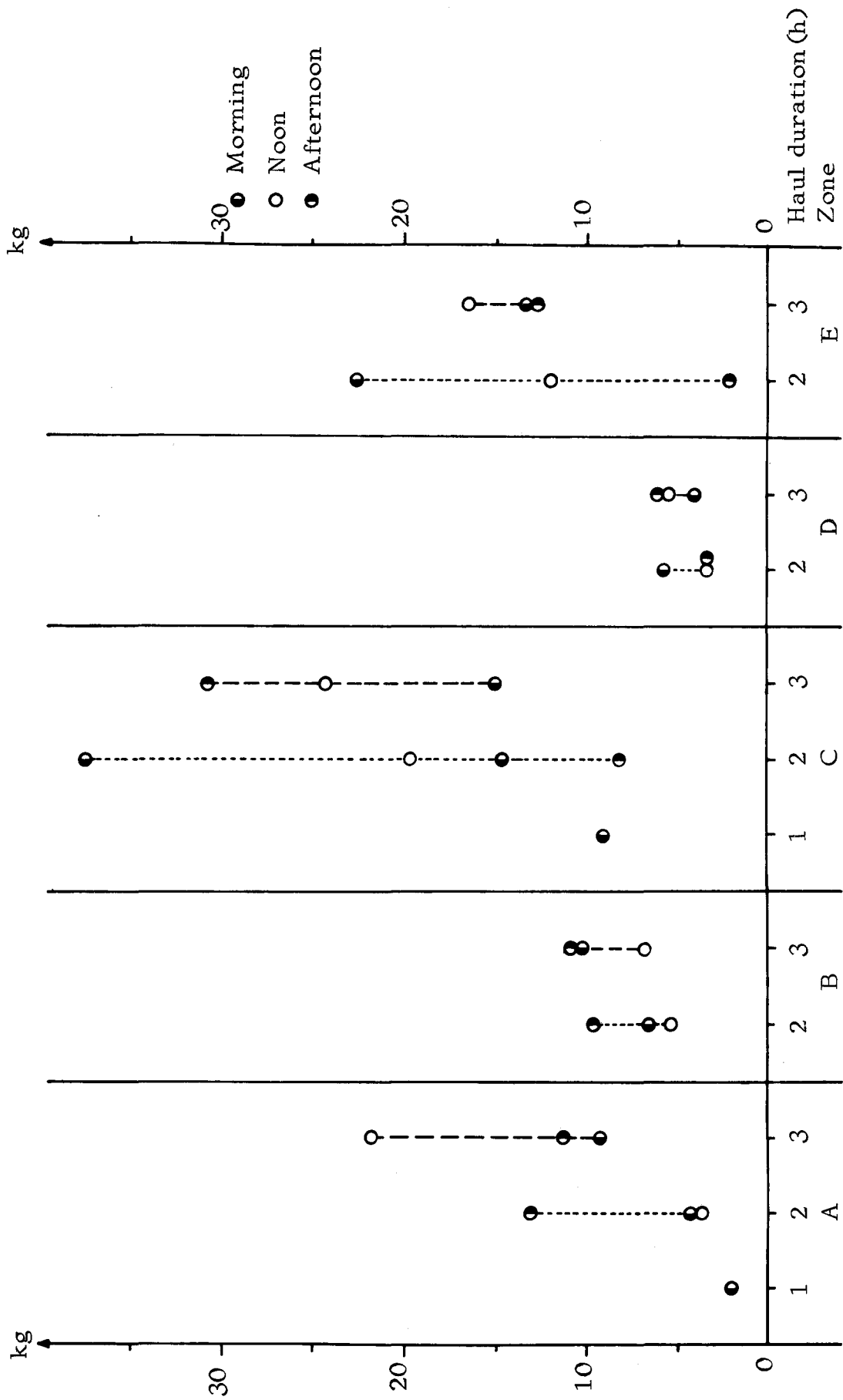


Fig. 15

Variability of catches taken with a bottom trawler in summer 1967

(weight totals per haul).

TABLE 4

List of the more important species

(can occur in considerable quantities in the trawl catch)

Pelagic

Engraulis encrasicolus T./

Trachurus trachurus and/mediterraneus

Sardina pilchardus

Gadus poutassou

Loliginidae

Sepiolididae

Demersal

Merluccius merluccius

Boops boops

Pagellus erythrinus

Diplodus annularis

Maena chryselis

Phycis blennioides

Capros aper

Sepia officinalis

Bottom

M./
Mullus barbatus and/surmuletus

Conger conger

Trachinus draco

Gobius niger

Arnoglossus laterna

Triglinidae

Solea vulgaris and S./lascaris

Solea lutea

Gaidropsarus biscayensis

Cepola rubescens

Raja asterias

Octopus sp.

Eledone sp.

Squilla mantis

Penaeus kerathurus

Uranoscopus scaber

bottom fish decreases showing the influence of haul duration for these groups (Fig. 16).

Similar results were obtained for zone C (Fig. 17).

From the fish caught, length distributions were plotted. An example is given in Fig. 18. As can be seen from the figure, the size distribution in the zones is different. Large specimens are mainly caught in zone C (330 m).

From a few of the predominant species scales and otoliths for age determinations were collected, but it is not yet possible to determine the actual age of these fish.

Extending the investigations on the influence of time of day on catches, two 24^h excursions (24/25 October and 13/14 November) to zone S (25 m depth) were made. From noon to noon seven hauls of three hours duration were carried out.

Although the total catch per haul (in individuals and in kg as well) reaches its peak on the 24/25/X at night and on the 13/14/XI by daylight, a clear influence of the time of day can be recognized when the catch is divided into fish occurring in greater quantities during the night and others caught mainly by day or at twilight (Fig. 19).

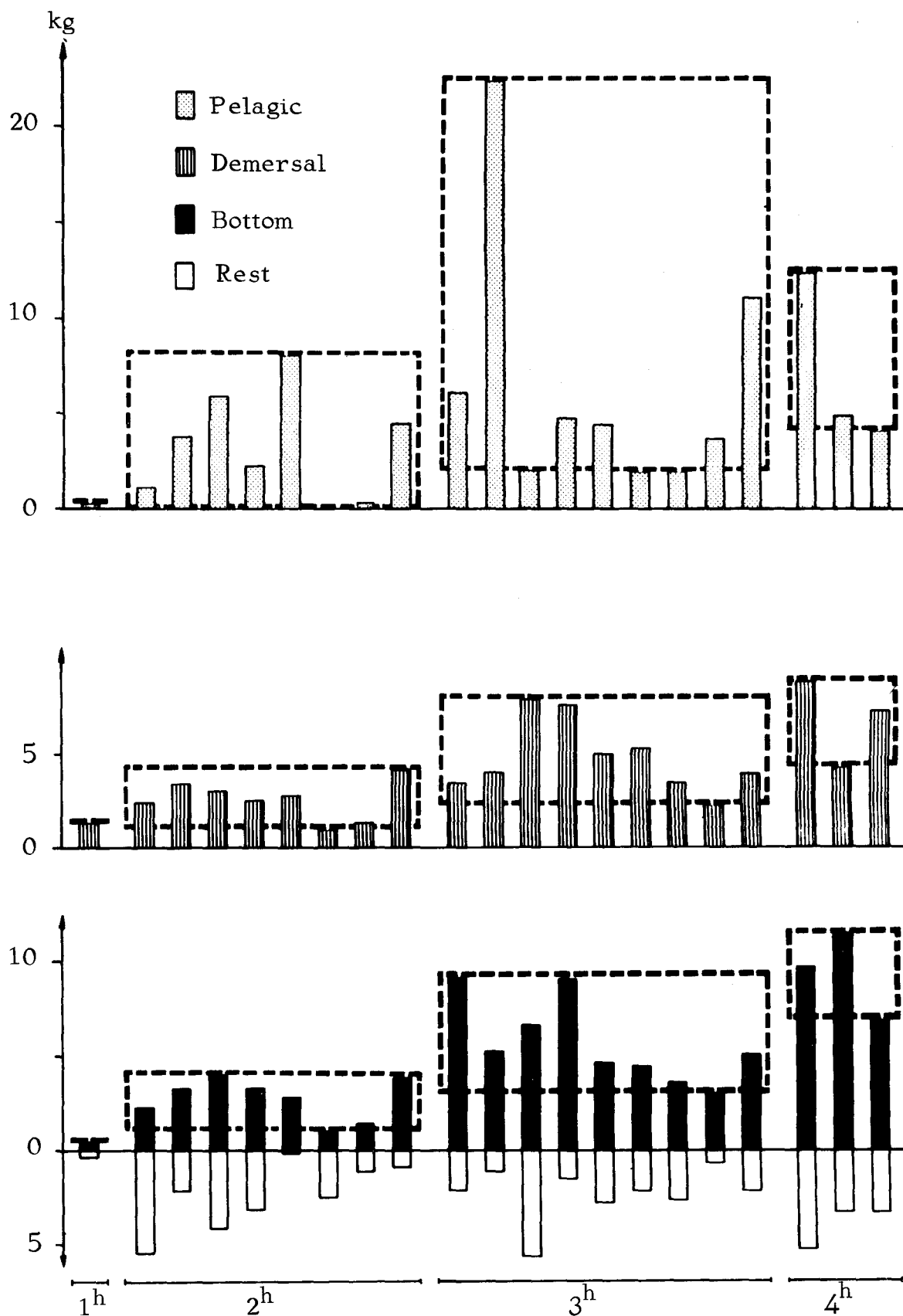


Fig. 16

Variability of trawl catches in zone S/in summer 1967.

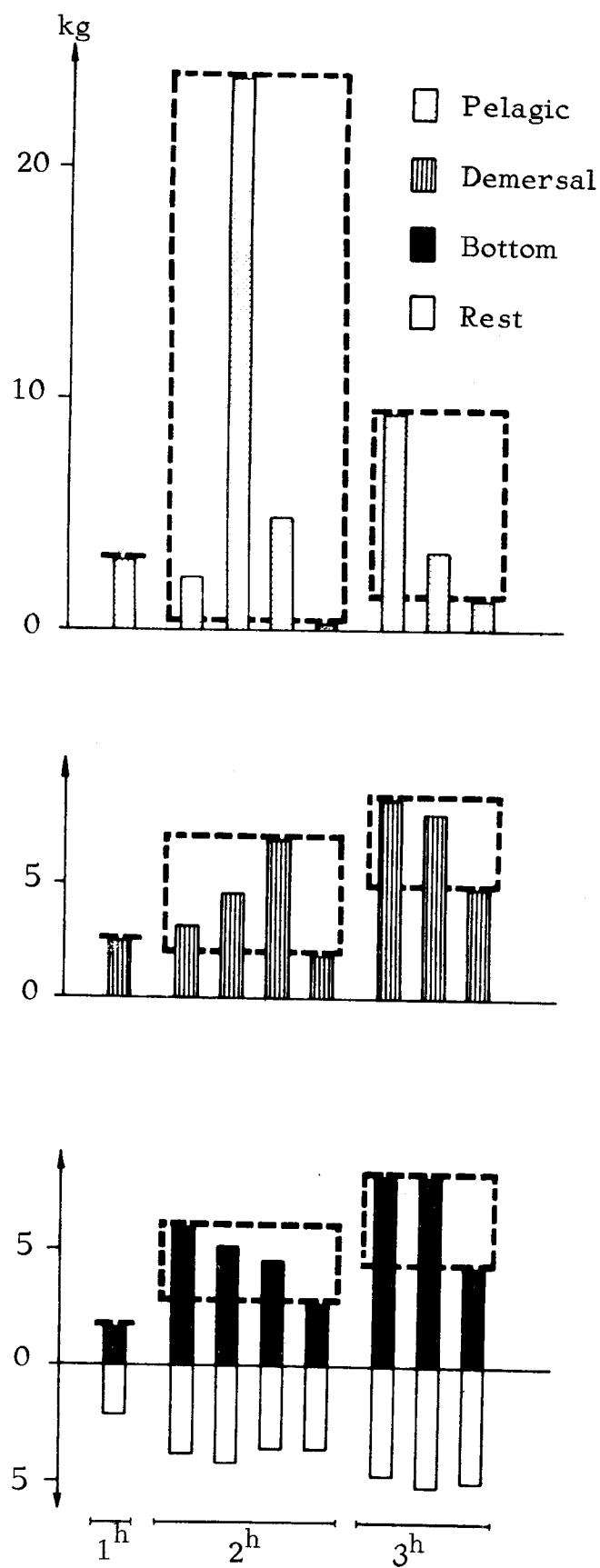


Fig. 17

Variability of trawl catches in zone C/ (330 m depth) / in summer 1967.

Number of
Individuals

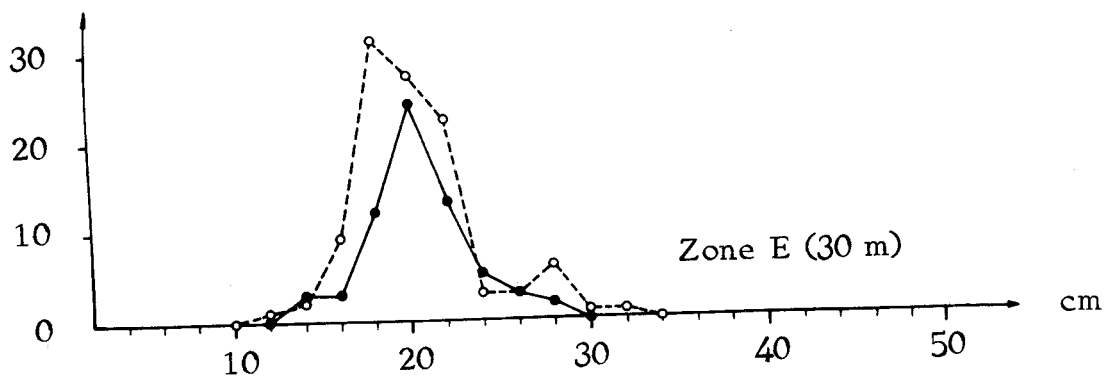
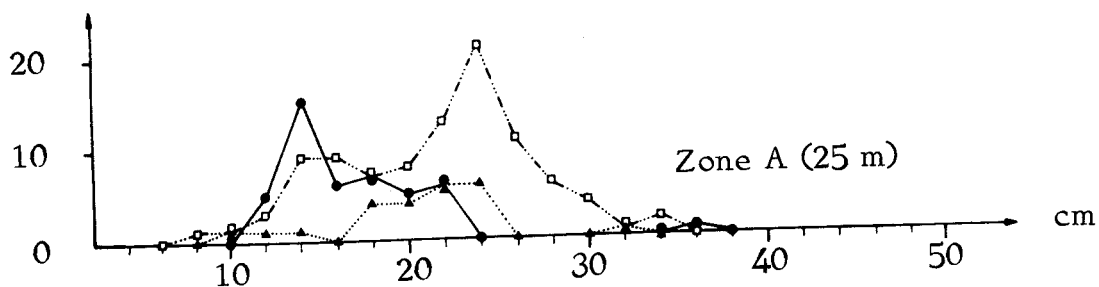
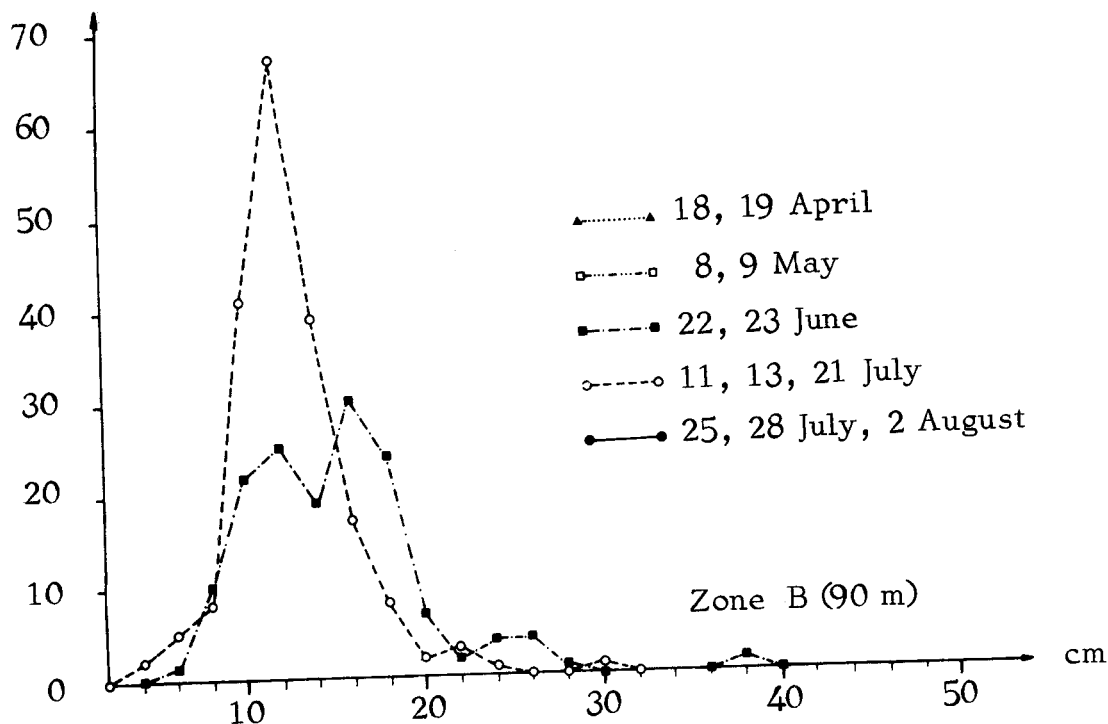
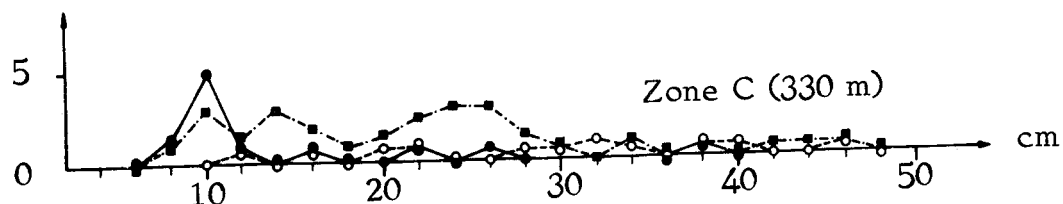


Fig. 18

Length distribution of hake (*Merluccius merluccius*) in different zones.

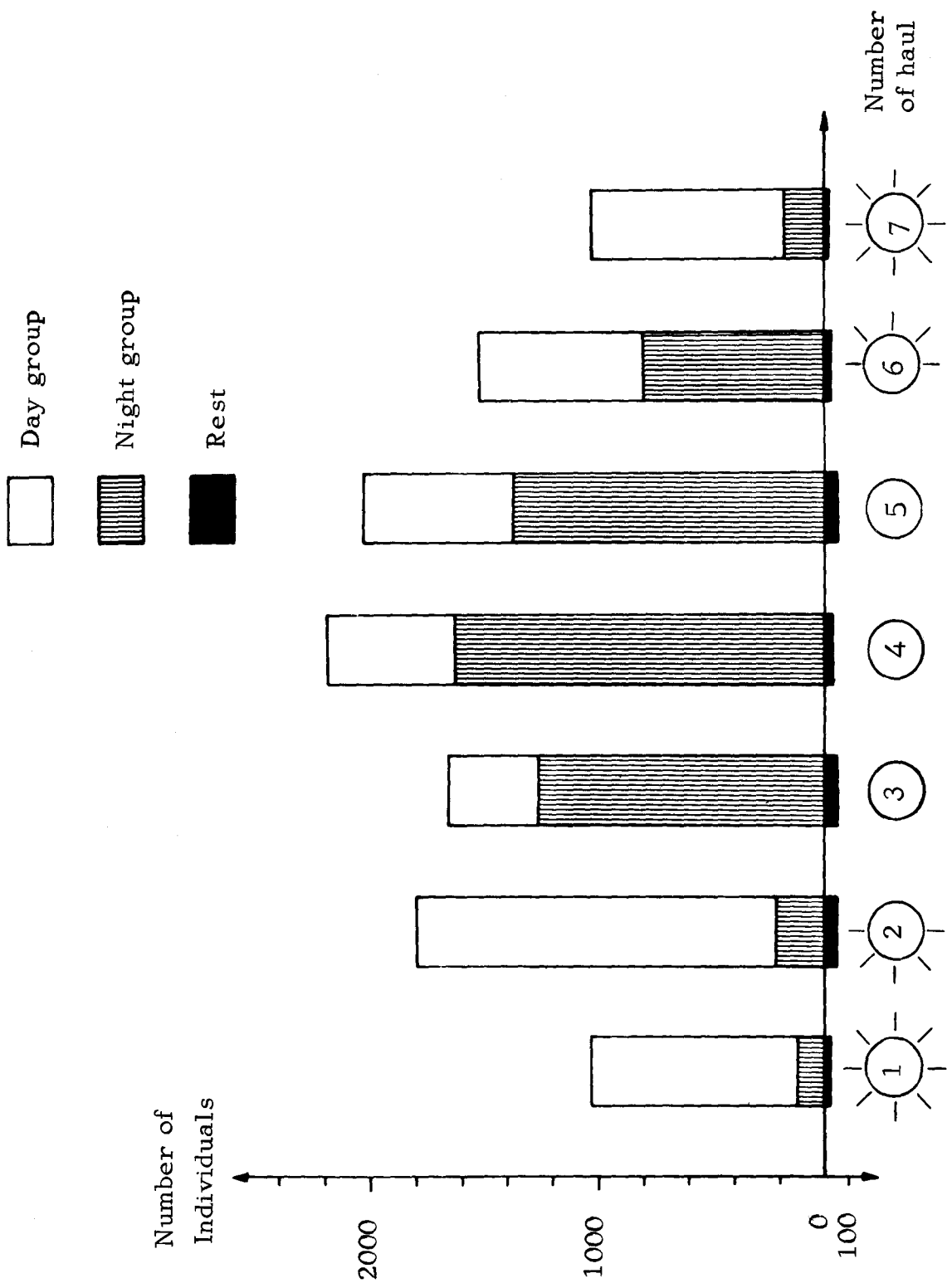


Fig. 19

(24/25-X-1967)/

Diurnal variation of the catch in zone S/. Each column represent one haul of 3 hours duration, the first and the last haul being made at noon and haul n. 4 at mid-night.

More important species of the day group are:

Mullus barbatus, Loliginidae, Pagellus erythrinus, Engraulis
encrasicholus and Maena sp.

The night group consisted mainly of:

Arnoglossus cf. laterna, Sepiolinidae, Squilla mantis, Sepia
officialis, Conger conger, Gobius niger and Eledone sp.

In order to obtain a basis for the calculation of the fishing effort, the
participation/^{in fishing/}of the fishing boats of a small port was registered by daily
identification of the individual boats present in the harbour.

Calculation for eleven boats of different sizes shows that the average
duration of absence from the harbour for fishing per boat and fishing day
in October, for example, was more than 11 hours, but less than 9 hours
and half in December.

SPECIAL DEVELOPMENT GROUP

The general trend in oceanography and marine ecology points to the direct measurement of environmental parameters and their continuous recording in space and time. Apart from permitting repetition of dubious measurements, and making the data immediately available, the in situ techniques make it possible to determine whether the samples undergo changes after collection.

In order to make in situ colorimetric determination possible, an automatic under water analyzer, similar to the "Technicon Autoanalyzer" was developed, which can be lowered to depth down to 300 m and transmits the information data to the ship via a multicore cable.

The underwater unit consists of two metal containers connected to each other by a flexible tube and maintained at about 2 atm over the hydrostatic pressure of the surrounding seawater a compressed air by means of control system (Fig. 20).

The control system consists of pressure regulators (E_1 , E_2 , D, C) similar to those used on SCUBA equipment. All the waste is collected in special containers (waste), where it is retained, so that the surrounding water will not be contaminated.

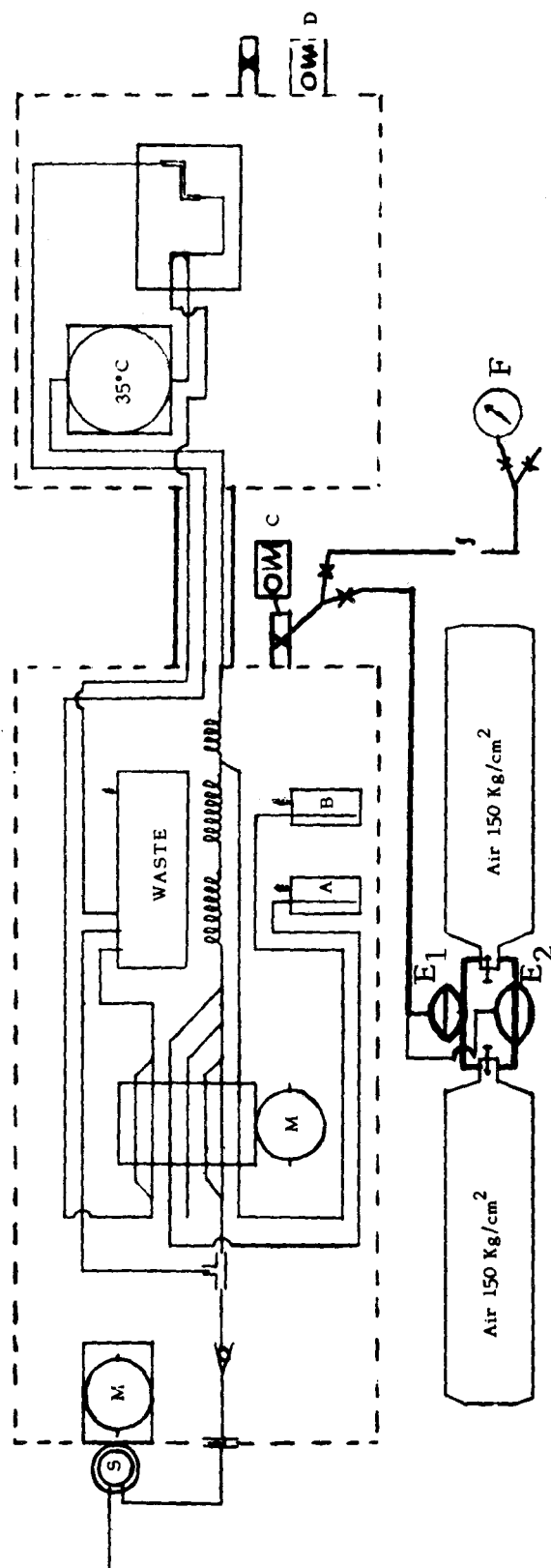


Fig. 20

General scheme of the under water unit of automatic analyzer.

The continuous analysis may be carried out for certain periods of time.

Therefore it was necessary to install a device (L) which would permit us to check from time to time the base line and at least one standard.

The device L can be actuated from aboard and will go through the following cycle:

- 1) deviation of seawater samples into "wastes";
- 2) introduction of the blank solution for 3 min into the Autoanalyzer;
- 3) introduction of a standard for 3 min;
- 4) return for 3 min to blank solution;
- 5) finally return again to the introduction of the seawater samples into the autoanalyzer.

The water sample is collected with a plastic peristaltic pump (S) driven by the a.c. electric motor (M), which is also able to pump water into the container at a pressure difference of more than 7 atm without a major variation in the flow rate.

A part of the water sample is drawn at (H) and the water sample is then mixed with reagents (e.g., A and B) according to the normal Autoanalyzer technique.

The two metal containers are held together in a large hydrodynamic casing made of glass-reinforced polyester, into which the two air tanks are also inserted (Fig. 21).

The unit is connected to the ship by a coaxial cable. The cable supplies the electrical energy for the underwater apparatus, can be used for carrying out certain changes in the analysis pattern and brings aboard the filtered signals for the recorder and for the determination of the internal pressure.

Fig. 22 shows a plotting obtained at sea.

An apparatus is under development which will permit the automatic separation of ions from seawater with the aid of ion exchange resins.

The apparatus consists of two sample changers similar to the previously developed sample changer for one bottle (one bottle sampler).

The new samplers, using a different technique, can go through a cycle of opening, collecting a sample and closing five bottles simultaneously with stoppers. This cycle is part of a larger cycle which will permit the separation of substances from a seawater sample by passing the sample through a heated ion exchange column, washing this column,

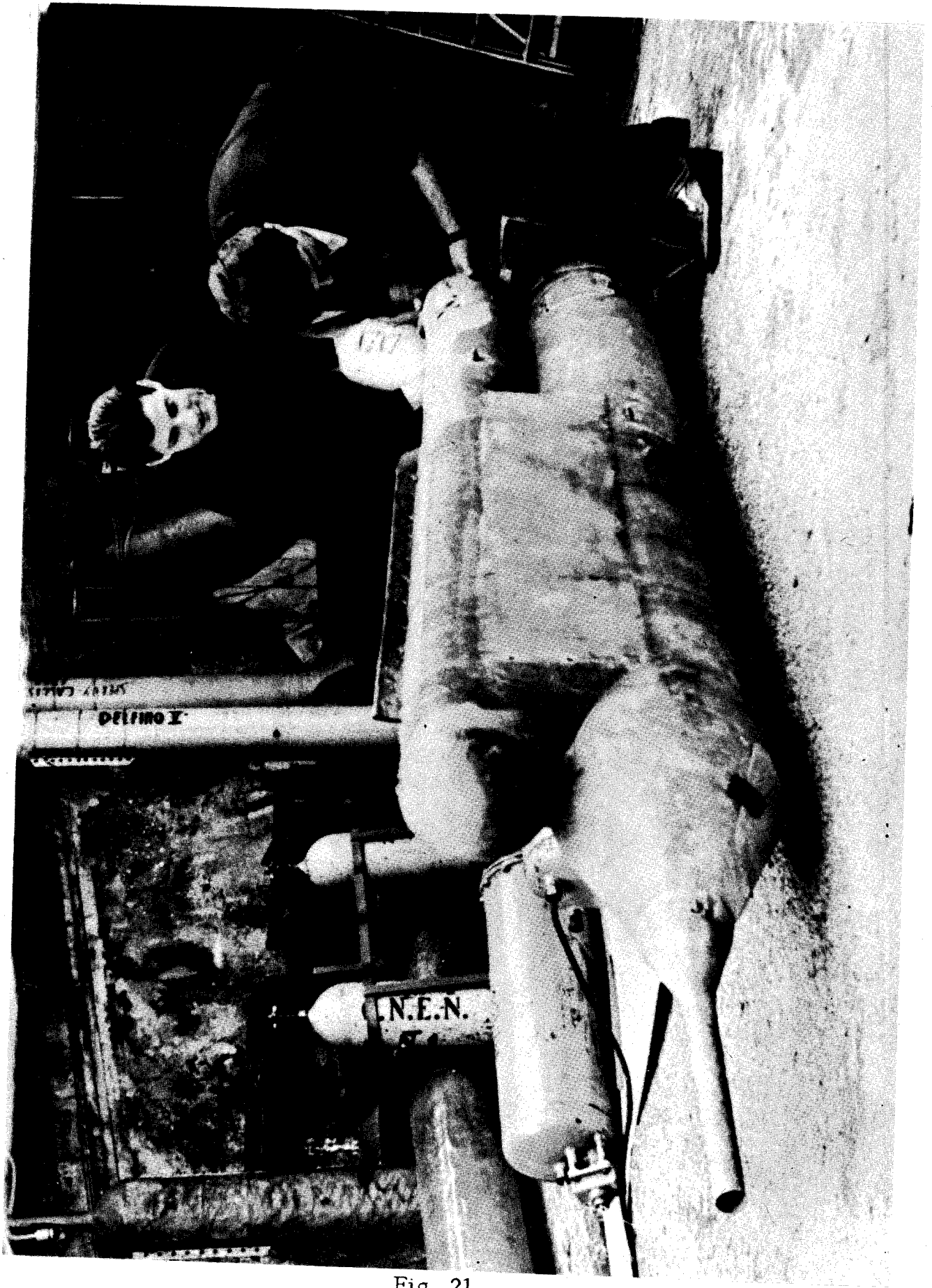


Fig. 21

The underwater unit of the automatic analyzer.

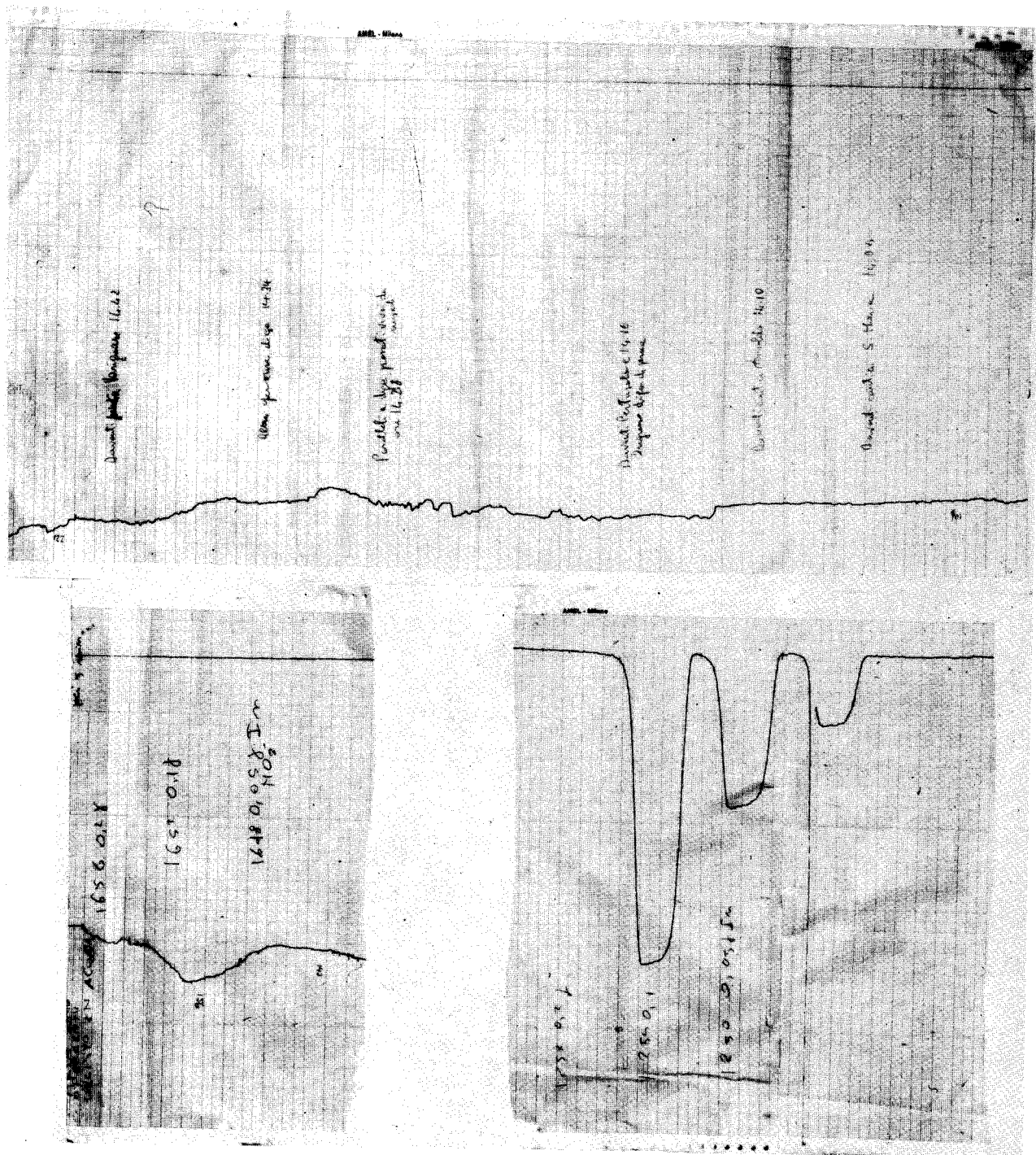


Fig. 22

Registration with underwater automatic analyzer obtained at sea.

eluting it, washing it again, rechanging it and finally washing the column again.

The F + M gas-chromatograph has been modified, so that it is now possible to register automatically the activity of the ^{14}C in the sample together with the stable C, N and H.

Several items of minor importance have been constructed or adapted e.g., one depressor with a build-in hydrophone, four new high speed plankton samplers complete with electrical flowmeter, one 'pinger', to be used for exact depth measurement near the bottom of the sea, etc.

During this period the "Odalisca" has been out for 35 one-day cruises, five of which in collaboration with Prof. B. Schreiber of Parma, and the trawler "S. Gabriele" for 22 one-day fishing trips.

COLLABORATION AND VISITS

Collaboration with Prof. B. Schreiber, of the Zoological Institute of the University of Parma, continued during 1967 as in previous years.

The eighth Contact Group Meeting was held at Fiascherino on 4-6 June 1967, and was attended by: Dr. R. Amavis, Directorate for Radiological Protection, EURATOM, Brussels; Dr. P. Bourdeau, Divisione di Biologia, EURATOM, Ispra; Dr. V.T. Bowen, Woods Hole Oceanographic Institute, Woods Hole (USA); Dr. M. Branica, Dept. of Physical Chemistry, Institute Ruder Boskovic, Zagreb; Dr. F. Breuer, Divisione di Protezione Sanitaria, CNEN, Roma; Dr. E.K. Duursma, International Laboratory of Marine Radioactivity, Monaco P.; Dr. M. Istin, Groupe de Biologie Marine (CEA) Station Zoologique, Villefranche-sur-mer, Alpes Maritimes, France; Prof. J. Joseph and S. Keckes, International Laboratory of Marine Radioactivity, Monaco P.; Prof. P. Korringa, Rijksinstituut voor Visserij Onderzoek, IJmuiden, Holland; Dr. A. Locker, Institut für Strahlenschutz, Reaktorzentrum, Vienna; Dr. Lopechon, International Laboratory of Marine Radioactivity, Monaco P.

Prof. F. Möller, Istituto di Statistica, Università Bocconi, Milano;

Prof. G. Montalenti, Istituto di Genetica, Università di Roma;

Prof. C. Polvani, Settore Radiazioni, Divisione di Protezione Sanitaria
e Controlli, CNEN, Roma; Dr. V. Pravdic, Dept. of Physical

Chemistry, Inst. Ruder Boskovic, Zagreb; Prof. C. Triulzi, CISE

(Centro Informazioni Studi Esperienze), Milano; Dr. O. Signorini,

CNEN, Roma; Dr. F. Van Hoeck, Directorate for Biology, EURATOM,

Brussels; Dr.ssa L. Forti, CNEN, Roma.

In addition to the persons mentioned above the laboratory was visited during 1967 by the following persons:

- Dr. G. CALDERALE - Segretario Generale del CNEN Roma
- Prof. C. POLVANI - Direttore Settore Radiazioni - Divisione di
Protezione Sanitaria e Controlli - CNEN Roma
- Dr. E. CITTERIO - Direttore Divisione Amministrativa - CNEN Roma
- Dr. A. BERG - Servizio di Biologia EURATOM - Ispra
- Dr. D.M. DANKAR - Smithsonian Institution - Director Mediterranean
Marine Sorting C. + Inst. Nat. Oc. et P. Tunisia
- Prof. FEDERICI - USEA, S. Terenzo, La Spezia
- Dr. F. GABRIELLI - Istituto Chimica Biologica - Pisa

Dr. P. KRAWARIK - Impulsphysic GmbH - 400 Sülldorfer Landstrasse
2 Hamburg 56 - Rissen

Dr. D. LAUSI - Istituto Botanico - Università di Trieste

Dr. E. LOCARDI - Laboratorio Geominerario CSN, Casaccia - Roma

Dr. G. LUGETTI - Istituto Zoologia e Biologia Generale, Università
di Pisa

Dr. P. LUPORINI - Istituto di Zoologia Università di Pisa

Dr. Ing. M. MACELLAIO - Sindaco di Lerici (Lord Major of Lerici)

Dr. Ing. M. MAGNAGO - Consulente Gabinetto Ministro Ricerca
Scientifica, Via Savoia 44 - Roma

Dr. M. MAZZA - Istituto Biologia e Anatomia Comparata
Università di Pisa

Dr. M. MERLINI - Servizio di Biologia, EURATOM - Ispra

Dr. A. MERLINI - Stato Solido Fisico, EURATOM - Ispra

Prof. M. MITTEMPERGER - Direttore Laboratorio Geominerario
CSN Casaccia - Roma

- Prof. R. NOBILI - Istituto di Zoologia, Università di Pisa
- Prof. F. PAPI - Istituto di Zoologia e Biologia Generale,
Università di Pisa
- Dr. A. PIGNATTI - Istituto Botanico, Università di Trieste
- Dr. O. RAVERA - Servizio di Biologia - EURATOM - Ispra
- K. SZEKIELDA - Station Marine d'Endoume et Centre d'Océanographie
Rue de la Batterie des Lions - Marseilles(7^e)
- Dr. R.A. VALLENVEIDER - Istituto Italiano di Idrobiologia
Verbania - Pallanza, Novara
- Dr. N. YAMAGATA - Nuclear Safety Res. Association
of Japan, Minato-ku-Tokio

During 1967 Dr. M. Bernhard attended the ENEA Meeting:

"Esperimento scarichi radioattivi nell'Atlantico", Lisboa, April 1967;

the second "National Symposium on Radioecology" and the "Conference

on System Analysis for the International Biological Program", both

held at Ann Arbor in May, 1967; during the voyage he visited the

Institute Marine of Science, Miami; Puerto Rico Nuclear Centre; Scripps

Institution of Oceanography, California; Haskins Laboratories, New York.

Dr. Bernhard also participated in the "Third International Colloquium

on Medical Oceanography", Nice, September 1967; and the "Comité

Consultatif de Biologie", Brussels, October 1967.

Dr. E. Torti participated the Technicon Symposium "Automation in

Analytical Chemistry", Brighton, November 1967, and visited Dr. Riley

at the University of Liverpool.

Dr. M. Bernhard, Dr. E. Torti, Dr. A. Zattera, Dr. C. Peroni and

Dr. M. Verzi, participated the Ninth Contact Group Meeting which was

held at Rovinj and Zagreb on September 1967.

REPORTS AND PUBLICATIONS PREPARED IN 1967

RTI/LCM (67) 1

BERNHARD M. - Studies on the radioactive contamination of the sea.
Annual Report, 1966.
CNEN Rep. (67)35 - EUR 3635e

RTI/LCM (67) 2

BERNHARD M. and A. ZATTERA - A comparison between the uptake of
radioactive and stable zinc by a marine unicellular
algae.
(To be published in the Proc. of Radioecology
Symp., Ann Arbor, May 1967).

RTI/LCM (67) 3

BERNHARD M. - Laboratorio per lo Studio della Contaminazione
Radioattiva del Mare.
Review of published achievements during 1963-1967.
(Internal report).

RTI/LCM (67) 4

BERNHARD M., E. TORTI and G. ROSSI - Automatic determination of
total hydrolyzable $P=PO_4^{---}$ in seawater and algae
cultures.
In: Automation in Analytical Chemistry.
Proc. Technicon Symp., Brighton, Nov. 1967, 395-400
Ed: E. Kawerau, Mediad Inc. New York (1968)

RTI/LCM (67) 5

TORTI E. and C. PAPUCCI - Determination of total Zn in seawater
with the resin chelating agent CHELEX-100.

RTI/LCM (67) 6

TORTI E. and C. PAPUCCI - Possibilities of metal complexation in
seawater.

RTI/LCM (67) 7

BERNHARD M., L. RAMPI and A. ZATTERA - Technical report.

RTI/LCM (67) 8

BERNHARD M., E. TORTI, G. ROSSI, M. GHIBAUDO and A. BRUSCHI -

An under-water apparatus for the in situ determination with the autoanalyzer technique.

In: Automation in Analytical Chemistry.

Proc. of Technicon Symp.,

Brighton, November, 1967, 391-394

Ed: E. Kawerau. Mediad Inc. New York (1968).

RTI/LCM (67) 9

MÖLLER F. and C. PERONI - An application of numerical classification of marine organisms.

Quality and Quantity. Eur. J. of Methodology, 2
n. 1-2, Jan. 1968.

RTI/LCM (67) 10

BERNHARD M. - Research on the metabolism or the fixing of some radioactive elements in the marine environment

(To be published in the Proc. of the III Intern.
Coll. on Medical Oceanogr., Nice, September, 1967).

RTI/LCM (67) 11

RAMPI L. and M. BERNHARD - A simple key for common mediterranean

Diatomeae, Peridinieae and Coccolithophorideae.

A simple tool for the identification of species in
production and radiocontamination studies.

(Pubbl. staz. zool. Napoli - in press).

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e Anatomia Comparata, Università di Parma (Italy)
Member for CNEN

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Member for CNEN

Dr. F. VAN HOECK - Biology Division, European Atomic Energy
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Member for EURATOM

Secretary:

Dr. L. FORTI - Settore Radiazioni, CNEN, Roma (Italy).

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Direttore: Dr. Michael Bernhard

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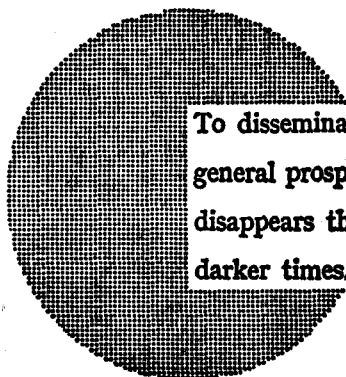
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Alfred Nobel

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